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OF
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January, 1942

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THE DISTRIBUTION OF CERTAIN OXIDATIVE ENZYMES IN THE CHORIOID PLEXUS[1, 2]

JONAS S. FRIEDENWALD, HEINZ HERRMANN AND ROBERT BUKA

From the Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital

Submitted for publication November 28, 1941

The present investigation is the outgrowth of studies on the mechanism of formation of the intraocular (1) and cerebrospinal fluids (2). The probable sources of these fluids are the processes of the ciliary body in the eye and the chorioid plexus in the brain. These organs are composed of a loose and very vascular connective tissue stroma covered by a single or double layer of epithelial cells. In the previously reported studies we have concerned ourselves in the first instance to discover a possible source of energy in the tissue which might be available for secretory work. Such a possible source of energy was found in a difference between the redox potential of the epithelium and that of the stroma in these tissues, the epithelium maintaining an oxidizing potential, as measured by redox indicator dyes, approximately $\frac{1}{4}$ volt more positive than the stroma. Furthermore a group of reversibly oxidizable substances were found to exist in the space between the epithelial and stroma cells, capable of mediating redox interaction between these two tissues.

Since the metabolites of the stroma and epithelium are in all probability identical, the cause of the difference in potential is to be sought for in their enzyme systems. In the present study we propose to investigate this aspect of the problem directly. That the respiratory metabolism of these tissues is not trivially small was already indicated by the work of Krebs (3) who found that the metabolic rate of the rabbit chorioid plexus was equal to that of the kidney, and greater than that of the brain.

[1] This investigation was supported in part by the John and Mary Markle Foundation.

[2] A preliminary report of this work was presented at a meeting of the American Society of Biological Chemists in Chicago, April, 1941.

EXPERIMENTS

The first problem which we encountered was the technical one of separating the tissue components. It was soon found that by shaking and stirring the tissue in certain bathing fluids, the epithelium was desquamated. Pure preparations of the epithelium were thus obtained with ease. Some epithelial cells or cell fragments cling to the stroma yielding a slight contamination in the preparation of this tissue. If physiological salt solution or neutral phosphate buffers are used for the bathing fluid, the histological structures of the separated tissues remain fairly intact but certain enzymes that are not firmly attached to the cell structures are lost in the bathing fluid. Choline esterase is an example of such a readily extracted enzyme. If a saturated solution of sodium sulphate is used, the enzymes are well retained in the tissues but may become inactivated through loss of coenzymes or mediators. In testing the distribution of a particular enzyme it was necessary, therefore, to be certain that the full activity of the whole undisturbed tissue was recovered in the sum of the activities of the separated components either with, or without, the addition of the appropriate coenzyme or mediator.

Material

The material for study was obtained from the brains of freshly slaughtered pigs.[3] The plexus was readily exposed by removing the upper and medial walls of the lateral ventricle. Its attachments and larger vessels were sectioned in the neighborhood of the foramen of Monroe and the tissue removed. The material obtained from one lateral ventricle will be referred to as one plexus. The wet weight per plexus was usually between 120 and 150 mgm., but occasional organs weighing as little as 80 mgm. were encountered. Dry weights averaged 16–17 per cent of the fresh wet weights. The epithelium accounts for roughly one third of the total dry weight, blood within the stroma vessels for one third, stroma tissue for the remaining third. When a plexus is laid smoothly on a glass it forms a roughly rectangular mass approximately 60 × 10 × 0.2 mm.

[3] We wish to express our gratitude to the Schluderberg-Kurdle Corp. for their generous help in supplying us with the required tissue.

Cell Counts

The epithelial cells separated from the stroma in Ringer's solution form a well dispersed suspension and may be stained and counted in an ordinary blood counting chamber. An estimate was thus obtained of approximately 10,000,000 epithelial cells per 100 mgm. of plexus. Allowing 30 mgm. for the wet weight of the epithelium, this gives a weight of 3×10^{-6} mgm. per cell which accords well with the average dimensions of these cells of slightly over 10 microns (3). The stroma was washed in distilled water until no further protein was extracted. It was then mounted on a slide, fixed and stained. Photomicrographs were taken of sample areas of the stroma and the number of cells counted in the photographs. From these it was estimated that the stroma contains 4,000,000 cells per 100 mgm. of plexus; approximately half of the cells constitute the walls of capillaries and small blood vessels. Since the volume of the individual stroma cells is much less than that of the epithelial cells this estimate accords well with the fact that by far the major volume of the stroma is extracellular fluid. These data were in agreement with the distribution between epithelium and stroma of "nucleic acid phosphorus" (total phosphorus after extraction with 2 per cent acetic acid and alcohol ether).

Separation of Epithelium and Stroma

The method of separation in saturated sodium sulphate solution will be described in detail since the greatest number of difficulties were encountered with this separating agent. Weighed amounts of tissue were suspended in a solution of sodium sulphate saturated at 35°C., 10 cc. of solution per gram of tissue. The suspended tissue was then stirred with a glass hook rotating the hook back and forth at the rate of about 2 full turns per second. The vessel containing the tissue should be as small as possible; for example, with tissue amounts up to 0.5 gm. we used 5 cc. bottles. During this stirring the vessels were submerged in a water bath at 38°C. so that the sodium sulphate would not crystallize. The fluid soon became cloudy with desquamated epithelium. After 10 to 15 minutes stirring the cloudy fluid was decanted from the stroma and filtered through Whatman No. 1 paper at 38°C. After decantation only as much solution was added to the

remaining stroma as was necessary to cover it, and the stirring and decanting were repeated. This procedure was continued until the decanted fluid was clear and practically free of epithelium. With sodium sulphate solution many washings were required to obtain approximately complete separation. One gram of tissue required about 3 to 4 hours washing, while larger amounts took still longer. With fluids such as trichloracetic acid or picric acid, tap water and distilled water, complete separation of epithelium and stroma was reached much sooner. The last excess fluid was removed from the stroma by gently pressing the tissue between filter paper. The separated tissues were now ready for experimentation or for storage at 0°C. until needed.

Spontaneous Respiration

These measurements were made manometrically with the Warburg apparatus. With freshly removed pig's plexus suspended in phosphate buffer at pH 7.4 we found a Q_{O_2} of 9–12. This is considerably lower than Krebs' figure of 19 for rabbit plexus in serum. Approximately 75 per cent of the spontaneous respiration is suppressed by cyanide. If the whole plexus is ground vigorously in a mortar for 3 minutes, the spontaneous respiration is almost completely lost.

Cytochrome Oxidase

Previous studies on the chorioid plexus have shown that "indophenol oxidase" was present in high activity in the epithelium but was absent or extremely feeble in the stroma. These studies were based on the use of the nadi reaction (dimethyl-paraphenylene-diamin and alpha naphthol). Since the presence of a strong reaction in the epithelium obscures the picture in the stroma, we repeated this test on stroma washed free of epithelium. No color formation takes place in the stroma proper, but a small amount of indophenol blue precipitate develops in the muscular wall of the larger arteries. The separated epithelium gives a strong color.

The activity of cytochrome oxidase in the tissue was tested manometrically with the Warburg method based on the investigations of Stotz and his collaborators (4) (5) comparing the activity of the whole tissue with that of separated epithelium and stroma. Tissue samples

of about 0.3 gm. fresh weight, or the separated epithelium and stroma from such an amount of fresh tissue were separately ground vigorously in a glass mortar, suspended in 2 cc. M/15 phosphate buffer at pH 7.4 and pipetted into the main compartment of the Warburg vessel. The side bulb contained 0.3 cc. N sodium succinate, or 0.3 cc. 2 per cent (para) phenylenediamin, or 0.3 cc. 2 per cent (para) hydroquinone. In some cases 0.5 cc. of a solution of cytochrome C prepared from heart muscle according to Keilin and Hartree (6) was added. The results are shown in Table I.

It was found in general that even with the gentlest separating procedure, some loss of activity occurred on separation, but with the less

TABLE I

Oxygen Uptake in cmm. per 100 mg. Chorioid Plexus in 20 Minutes at 38° C.

	SUCCINATE		PHENYL-ENEDIAMIN		PARA-HYDRO-QUINONE	
	O_2 uptake	Activity recovered	O_2 uptake	Activity recovered	O_2 uptake	Activity recovered
	mm.	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
Unseparated Control	40		48		7	
Epithelium	26	65	43	90	3	43
Epithelium and Cytochrome C	37	93	57	118	45	640
Stroma	1	5	1	5	0	0
Stroma and Cytochrome C	1	5	3	7.5	2	29

severe procedures (separation in Ringer's solution or in tap water) the full original activity could be recovered on addition of cytochrome C. During one brief period in the course of these investigations, the tissue consistently showed much greater resistance to the treatment, maintaining full activity after separation in the milder fluids and only slowly losing activity on prolonged treatment with sodium sulphate solution. During this period also maximal activity of the oxidase was observed yielding Q_{O_2} between 75 and 90 with succinate as substrate (Fig. 1) as calculated for the separated epithelium alone, and no increase in activity occurred on addition of cytochrome C. This is to be compared with Q_{O_2} between 50 and 70 obtained at other times. It would appear that during this period the cytochrome C content of

the tissue must have been either more abundant or more firmly held than at other periods. These observations were made in the autumn months but were not repeatable the following year. We have been

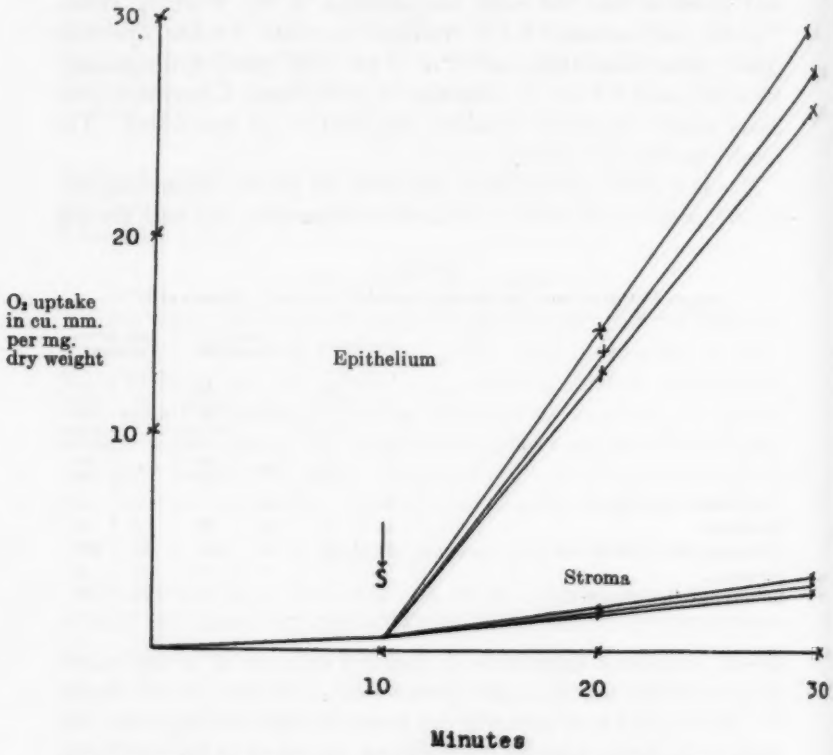

FIG. 1. SUCCINATE OXIDATION OF SEPARATED EPITHELIUM AND STROMA OF THREE SAMPLES OF CHORIOID PLEXUS

These results were obtained in the period when particularly active specimens were being studied (see text). Substrate added at S.

unable to trace the source or state of nutrition of the pigs that gave these unusual results.

No matter whether the original activity was recovered in the separated tissues with or without the addition of cytochrome C, upwards of

90 per cent of the total activity was accounted for by the epithelium. Since some epithelial cells and cell fragments cling to the stroma, it may be concluded that the epithelium accounts for almost the whole of the oxidase activity.

The inactivation of the oxidase system which occurs on exposing the tissue to saturated solution of sodium sulphate was studied more extensively. Samples of plexus were ground in a glass mortar and

TABLE II

Effect of Saturated Sodium Sulphate on Oxidase Activity

ORGAN	TIME OF Na_2SO_4 TREATMENT	OXYGEN UPTAKE IN CMM. OF 200 MGM. TISSUE IN 20 MINUTES						DECOLORIZATION TIME OF METHYLENE BLUE WITH SUCCINATE AS SUBSTRATE	
		Phenylenediamin as substrate			Succinate as substrate				
		Untreated control	Treated with Na_2SO_4		Untreated control	Treated with Na_2SO_4		Untreated control	Treated with Na_2SO_4
			With cytochrome	Without cytochrome		With cytochrome	Without cytochrome		
	minutes								
Plexus	½	90	50	20	—	—	—	—	—
Heart	20	—	107	30	150	88	16	—	—
Plexus	½	—	—	—	120	40	35	—	—
Plexus	½	156	89	40	100	42	14	—	—
Plexus	2	—	—	—	110	6	2	32	180
Plexus	2	90	52	26	123	8	6	30	150
Plexus	½	68	60	36	77	44	25	37	45
Plexus	½	45	52	11	33	30	6	42	43
Plexus	½	68	32	2	64	20	2	30	35
Plexus	½	52	48	6	51	32	7	32	38
Plexus	2	100	12	7	55	4	1	27	87

separated into several portions. One portion was tested directly for its oxidase activity with phenylenediamin or succinate. Other portions were placed in saturated sodium sulphate solution for varying periods and then poured into a larger volume of Ringers solution from which the tissue was recovered by centrifugation. Table II shows that after vigorous treatment with sodium sulphate the full original activity is not recoverable even on addition of an excess of cytochrome C. The irreversible inactivation, in general, is greater

in respect to succinate than in respect to phenylenediamin. This led us to test the activity for succinate by the Thunberg technique (see below) in which methylene blue acts as hydrogen acceptor for the succindehydrogenase succinate system to the exclusion of cytochrome C and of cytochrome oxidase. The results showed that succindehydrogenase activity is markedly reduced on exposure of the tissue to sodium sulphate for 2 minutes or more.

The progressive and step wise inactivation of the oxidative system by sodium sulphate is of some intrinsic interest. The first effect appears to be due to the loss of cytochrome C since the partially inactivated tissue could be reactivated by addition of cytochrome C. Furthermore, cytochrome C can actually be recovered in saturated sodium sulphate extracts of heart muscle. Inactivations of the succinate oxidizing system have recently been reported (7) (8). The compounds removed in those cases appear to have resembled protein and, therefore, are not likely to have been removed by the sulphate extraction. On the other hand, the possibility of the participation of metals in this oxidizing system has been shown (9) (10), and it may be that metal ions are lost in the extraction. The loss of activity could also be due to the formation of insoluble sulphates or to mild denaturation of the enzyme proteins. A possible clue to this problem is furnished by the much greater resistance of heart muscle oxidase than of chorioid plexus oxidase to inactivation by sodium sulphate.

Succindehydrogenase[4]

The epithelium was separated from the stroma in Ringer's fluid or tap water, or by very brief exposure to saturated sodium sulphate followed by tap water. Ground samples of whole plexus and of the separated tissues were placed in phosphate buffer at pH 7.4 with methylene blue in Thunberg tubes and the rate of decolorization of the dye was measured on addition of succinate under anaerobic conditions. (Tables III and IV.)

The succindehydrogenase activity was measured also manometri-

[4] Succindehydrogenase reduces cytochrome C through the oxidation of succinate to fumarate (1). Fumarate is converted into malate by fumarase through the addition of water (2). Malic dehydrogenase oxidizes malate to oxaloacetate (3) reducing the coenzyme diphosphopyridinenucleotide. Lactic dehydrogenase

cally by the method of Quastel (11). (Table V.) The results of both techniques indicated that the distribution of succindehydro-

TABLE III

Activity of Certain Dehydrogenases in the Chorioid Plexus as Measured with Thunberg Technique

Each sample contained 200 mgms. ground tissue, 5 cc. methylene blue 1:20,000, 0.5 cc. N substrate solution, 3 mgms. Cozymase, 0.05 N neutralized cyanide, N/20 phosphate buffer pH 7.7.

SUBSTRATE	DECOLORIZATION TIME
	minutes
Control	110
Succinate	30
Malate	5
Lactate	7
Glycerophosphate	40
Hexosediphosphate	30
Pyruvate	130
Glucose	90
Citrate	130
Oxybutyrate	90

genase was parallel to that of cytochrome oxidase, 90 per cent being located in the epithelium.

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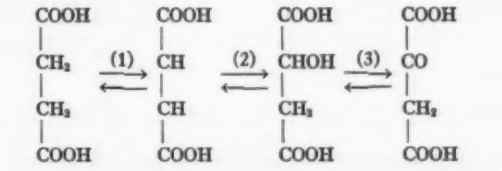
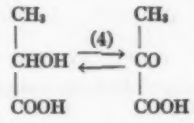
oxidizes lactate to pyruvate (4) also by reducing the same coenzyme. As indicated, the enzymes also catalyze the reactions in the opposite direction.



Succinic acid Fumaric acid Malic acid Oxaloacetic acid



Lactic acid Pyruvic acid

Fumarase

The separation of epithelium from stroma was carried out on 600 mgm. of tissue in sodium sulphate solution, it having previously been found that no loss of activity to this enzyme results from the procedure. The separated stroma and epithelium were each divided into three equal parts. One cc. of M/10 phosphate buffer pH 7.8 and 2 cc. of 0.4 M sodium fumarate were added to each sample. The

TABLE IV

Distribution of Dehydrogenases in Stroma and Epithelium of Choroid Plexus as Determined with Thunberg Technique

Reagents as indicated in Table III

SUBSTRATE	FRESH WEIGHT OF TISSUE	DECOLORIZATION TIME	
		Epithelium	Stroma
	mg.		
Succinate	500	7	95
Malate	100	14	40
Lactate	100	15	45
Hexosediphosphate	500	14	50

TABLE V

Distribution of Succindehydrogenase as Determined by the Method of Quastel

500 mg. tissue used for separation in sodium sulfate solution (½ min.) followed by Ringer's solution. The CO_2 output given is corrected for the output without addition of succinate.

	CO_2 OUTPUT	
	After 10 min.	After 20 min.
	cmm.	cmm.
Epithelium	189	342
Stroma	28	56

samples were incubated for various periods and the amount of malate produced was estimated polarimetrically following the prescription of Quastel (12). Ninety per cent of the fumarase activity was found in the epithelium. (Table VI.)

Various Dehydrogenases

The enzymes in this group are known to be unaffected by sodium sulphate and this separating fluid was therefore used. Cozymase

prepared from yeast was added when required. Activities were measured by the rate of anaerobic reduction of methylene blue according to Thunberg. The activity of lactic and malic dehydrogenases were determined also manometrically with methylene blue as carrier. Malic dehydrogenase activity was measured also by the Quastel (11) manometric method. The results of all three methods of estimation were in good agreement in spite of the fact that the

TABLE VI

Distribution of Fumarase

See description in text

	POLARIMETER READING IN 1/100 DEGREE		
	After 15 min.	After 30 min.	After 60 min.
Epithelium	18	62	98
Stroma	4	7	14

TABLE VII

Distribution of Malic-Dehydrogenase as Determined by the Method of Quastel

One gram of tissue was used for each separation. Separation fluid N/15 phosphate buffer pH 4.7. Duplicate experiments a and b are reported separately.

	CO_2 OUTPUT IN 20 MINUTES		RATIO $\frac{(2)-(1)}{(4)-(3)}$	
	a	b	a	b
	cmm.	cmm.		
(1) Epithelium	31	25		
(2) Epithelium + Malate	68	77	3.1	2.9
(3) Stroma	25	12		
(4) Stroma + Malate	37	30		

theoretical basis of the Quastel method is not clear in experiments in which the presence of cyanid is required.[5]

The results are summarized in the Tables III, IV and VII. Relatively large activities were found for lactic and malic dehydrogenase, lesser activities for hexose diphosphate and glycerophosphate, no activity was found with glucose, pyruvate or citrate as substrates. In contrast to the oxidase, succindehydrogenase, and fumarase, 90

[5] We are indebted to Dr. Louis Flexner for directing our attention to this point. His detailed discussion of this problem will appear elsewhere.

per cent or more of which was found in the epithelium, approximately 25–30 per cent of the activity of the dehydrogenases listed was present in the stroma. Since the epithelium contains approximately 2½ times as many cells as the stroma, it follows that the dehydrogenase activity of the stroma is as great per cell as that of the epithelium. In respect to malate and lactate, the activity of both these tissues is approximately as high per cell as that of the liver or kidney.

DISCUSSION

The experiments which we have reported constitute only a preliminary and exploratory study on the metabolism of the chorioid plexus. They suffice, however, to show that this tissue is in certain respects an ideal subject for quantitative enzymatic investigation. Some enzymatic activities encountered were very high, equalling those of the most actively metabolizing organs. The two major histological portions of the tissue, epithelium and stroma, are readily separable and a variety of different separating media have been found from among which a suitable one could be chosen for each of the enzyme systems so far studied. The whole organ is so delicate in structure that it can be treated in manometric procedures as if it were a tissue slice.

Though these studies furnish, we hope, merely the beginning of our investigation on the metabolism of this interesting tissue, they suffice to confirm completely the predictions based on measurements of the redox potentials of the tissue components with the aid of redox indicator dyes. This would appear important, for the well known tendency of dyes to capricious behavior in the presence of tissues has led some workers to mistrust any observations growing out of the use of indicator dyes in living tissues.

SUMMARY

The activity of respiratory enzymes in the chorioid plexus is intense. The cytochrome oxidase-succindehydrogenase-fumarase system is located almost entirely in the epithelial cells. On the other hand, the activity of malic and lactic dehydrogenase is as great per cell in the stroma as in the epithelium. These findings fully confirm conclusions previously drawn from a study of the redox potentials of these tissues as measured by redox indicator dyes.

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THE CHOLINE ESTERASE CONTENT OF THE CHORIOID PLEXUS AND CILIARY PROCESSES[1]

HEINZ HERRMANN, M.D., AND JONAS S. FRIEDENWALD, M.D.

From the Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital

Submitted for publication November 28, 1941

A series of studies on the mechanism of formation of the intraocular and cerebrospinal fluids has led us to the investigation of the enzyme content of the tissues which constitute the sources of these fluids, namely, the chorioid plexus and the ciliary body. In a previous paper (1) we have shown how the tissue components of the chorioid plexus, epithelium and stroma, may be separated from one another without destruction of their enzyme systems, and have reported on the activity of certain oxidative enzymes in the two tissue components. In the present study we have applied these separating techniques also to the ciliary processes.

Recent studies have suggested that the choline esterase content of various tissues may be correlated with their cholinergic activity (2, 3). Hellauer (4) denies any rigorous parallelism of this sort, and, while confirming the presence of high esterase activity in cholinergic organs, reports the presence of large amounts of the enzyme in adrenergic organs. The secretion of the intraocular fluid is apparently controlled by an adrenergic mechanism but there is some evidence that the vasomotor apparatus of this tissue is, at least in part, cholinergic. No data are available as to the humoral activators of the chorioid plexus.

EXPERIMENTS

Material was obtained from beef and pigs. The tissue was removed in the slaughter house and placed in small chilled glass bottles within half an hour of the animal's death. The chilled tissue reached the

[1] This investigation was supported in part by the John and Mary Markle Foundation.

laboratory within an hour of the slaughtering of the animals and was immediately worked up. Choline esterase is normally present in the blood serum and in some other extravascular fluids. Moreover, it is readily extracted from tissues on exposure to salt solutions of low concentration. It was necessary, therefore, to separate the epithelium from the stroma in the presence of a bathing fluid that would precipitate and fix the choline esterase in the tissue without denaturing the enzyme. The possibility that sodium sulphate solution might serve this purpose was tested on blood serum. To a small amount of serum a ten-fold volume of sodium sulphate solution saturated at 35°C. was added. The precipitate resuspended in Ringer's solution had practically the whole choline esterase activity of the original serum. The supernatant fluid freed of the greater part of its sodium sulphate by crystallization at 0°C. showed no activity. Samples of tissue similarly exposed to saturated sodium sulphate solution for several hours showed no loss of choline esterase activity.

The separation of epithelium from stroma was accomplished by agitating the tissue in saturated sodium sulphate solution. The details of the procedure are given in the previous paper. The separation of the epithelium from the stroma of the chorioid plexus is tedious but uncomplicated. In the case of the ciliary body some factors diminish the efficiency of the procedure. The careful excision of the ciliary processes avoiding inclusion of ciliary muscle and iris results in the extirpation of rather small pieces of tissue. Much patience is required to keep all these small fragments together when the flakes of epithelium liberated by shaking are poured off with the supernatant fluid. Furthermore, the epithelium of the ciliary processes sticks to the stroma more tenaciously than does that of the chorioid plexus, and the stroma of the ciliary processes is less resistant to mechanical strain. Thus the epithelial portion is finally contaminated with some shreds of stroma and some epithelial cells and cell fragments still cling to the stroma portion at the end of the separation. Nevertheless, a stroma fraction poor in epithelial cells and an epithelial fraction very poor in stroma elements can be obtained. From histological studies we estimate the mutual contamination of the two tissues as less than 15 per cent.

After the sodium sulphate treatment, the fluid was drained off as

thoroughly as possible and the tissue portions blotted dry with filter paper. Samples were ground vigorously in a glass mortar in 2 cc. of Ringer's solution containing 0.2 per cent sodium bicarbonate and transferred to the main compartment of a Warburg flask for esterase estimation according to Ammon's method (5). The side bulb of the Warburg flask contained 0.5 cc. of 2 per cent acetylcholin bromide solution. Controls were run with tissues not treated with sodium sulphate, and also with the reagents alone in order to estimate the amount of non enzymatic hydrolysis. We selected in the case of the chorioid plexus specimens which seemed on macroscopic examination to be practically free from blood. By rough estimation of hemoglobin content those specimens were found to contain less than 0.02 cc. of

TABLE I

Cholinesterase activity expressed in cu. mm. CO_2 produced in 20 minutes at 38 degrees, corrected for non-enzymatic breakdown

Tissue amount 300 mg. Epithelium/stroma ratio derived from results of activity measurements on epithelium and stroma separately, calculated for dry weight unit * or per total organ.

	PIG	BEEF
Ciliary Body:		
Total Activity	250, 280	54, 50
Epithelium/Stroma Ratio	1.5*	*1.3, *1.0
Chorioplexus:		
Total Activity	178, 185, 164, 171, 169	41, 36
Epithelium/Stroma Ratio	1.5, *1.3, *1.0, 1.5, 1.3, 1.1	*1.8, 1.6

blood. 0.2 cc. of pig's blood has almost the same cholinesterase activity as the stroma of one ciliary body. Consequently the blood contained in the tissue could account for less than 1/10 of the esterase activity found in the stroma. Specimens with engorged blood vessels contain up to 0.08 cc. blood, enough appreciably to affect the esterase activity measured. Hence those were discarded.

The results of the measurements are shown in Table I. Samples of chorioid plexus or ciliary processes from animals of the same species gave reasonably consistent results. Both organs in beef showed only 20–25 per cent of the activity of the same tissues in pigs. According to Glick (6), there is an enormous variation of esterase activity in different organs of the same animal, the parotid gland showing 100

times the activity of the least active organs. Compared with Glick's results, ciliary processes and chorioid plexus fall into the group of medium esterase activity, being approximately as active as the intestinal mucosa. It is interesting that in spite of this appreciable activity in these tissues and in spite of the fact that the enzyme is not firmly bound to cell structures, and is present in blood plasma and in some other extracellular fluids, only traces of esterase activity were found in the aqueous humor or in the cerebrospinal fluid (7, 8).

In both chorioid plexus and ciliary processes the activity of choline esterase in the stroma was almost as great as that in the epithelium. Allowing for the fact that there are two to three times as many epithelial as stroma cells in these organs, the activity per cell was greater in the stroma than in the epithelium. This is the highest relative enzyme activity of the stroma we have so far encountered. Possibly there may be considerable activity in the extracellular fluid of the stroma.

Flexner (9) has shown that the chorioid plexus of pig's embryos becomes of adult type and actively secreting when the embryos reach 45 mm. in length. We were interested to find out whether the choline esterase activity of the tissue changed markedly at this period of growth. For these minute organs the titrimetric micromethod of Glick (10) was used. No attempt was made to separate epithelium from stroma. Organs were obtained from embryos 30 mm. and 90 mm. in length. The activity of these tissues per milligram dry weight was not significantly different from that of adults. It follows that these experiments offer no grounds for suspecting that the secretory activity of the chorioid plexus is cholinergic.

SUMMARY

The chorioid plexus and the processes of the ciliary body show a moderate and similar choline esterase activity though there is no evidence that the secretory activity of either organ is cholinergic. The esterase activity of the stroma of these tissues is relatively high compared with that of the epithelium.

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p-AMINOBENZOIC ACID AS A METABOLITE ESSENTIAL FOR BACTERIAL GROWTH[1]

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Woods (1) has suggested that the sulfonamide drugs prevent bacterial growth by interfering with the metabolism of p-aminobenzoic acid. If this theory is to be substantiated, it must first be demonstrated that p-aminobenzoic acid is a metabolite essential for bacterial growth. Rubbo and Gillespie (2) have reported that minute amounts of this organic acid added to a synthetic medium stimulate markedly the growth of *Cl. acetobutylicum*. At the outset of the present investigation an attempt was made to repeat the experiments of Rubbo and Gillespie, but in spite of numerous trials their observation could not be confirmed. A further study was then undertaken of the role of p-aminobenzoic acid in bacterial metabolism and it was clearly shown that the compound, though ineffectual by itself, is extremely active as a bacterial growth factor in the presence of traces of biotin. On the basis of this finding it was concluded that p-aminobenzoic acid is a substance essential to bacterial metabolism and, therefore, is a compound which may well be involved in the mechanism of action of the sulfonamide drugs. The relation of p-aminobenzoic acid to the antibacterial action of sulfanilamide and its derivatives will be discussed in a subsequent paper (3).

MATERIALS AND METHODS

Cl. acetobutylicum, an anaerobic bacillus used in the commercial production of acetone and butanol, grows luxuriantly in a relatively simple synthetic medium provided certain specific growth factors are added (4). The basal medium consists of essential salts, glucose, and either asparagine or an ammonium salt as a

[1] This study was supported in part by a grant from the Rockefeller Foundation Fluid Research Fund.

source of nitrogen; the growth factors required include biotin and a chemical extract of yeast known as "B Y factor." Two strains of *Cl. acetobutylicum* were used in the present experiments; one was obtained through the courtesy of Professor W. H. Peterson of the School of Agriculture, University of Wisconsin, the other was secured from the American Type Culture Collection (no. 4259). The latter was one of the strains used by Rubbo and Gillespie in their experiments with p-aminobenzoic acid. Crystalline biotin[2] (5) was kindly supplied by Professor V. du Vignaud of the Cornell Medical School, and p-aminobenzoic acid was purchased from the Eastman Kodak Company.

Basal culture medium: The basal medium used was that described by Oxford and his coworkers (4) and had the following composition:

Glucose	20.0 gms.
Asparagin (or $(NH_4)2\ HPO_4$)[3]	1.0 gms.
K_2HPO_4	0.5 gms.
KH_2PO_4	0.5 gms.
$MgSO_4 \cdot 7H_2O$	0.2 gms.
NaCl	0.01 gms.
$FeSO_4 \cdot 7H_2O$	0.01 gms.
$MnCl_2 \cdot 4H_2O$	0.01 gms.
Reduced Fe	3–5 mgms.
Distilled H_2O	1000 cc.

The medium was tubed in 30 cc. lots in chemically cleaned 8 inch test tubes and was sterilized by steaming for 20 minutes just before use.

Cultural methods: Stock cultures of both strains of *Cl. acetobutylicum* were grown in basal medium enriched with peptone. The Peterson strain grew well in medium containing only 0.25 per cent peptone, whereas strain 4259 required 1.0 per cent peptone in order to attain satisfactory growth. Under these conditions both strains grew rapidly producing large amounts of gas. Growth was found to be maximum in 48 hours, at which time 10 cc. of the peptone culture was centrifugalized, the supernatant discarded, and the organisms resuspended in 5 cc. of sterile basal medium. 0.5 cc. of the suspension was used as a standard inoculum, for it was found after repeated failures that a large number of organisms was needed to initiate growth consistently in the basal medium despite the addition of the necessary growth factors. Following inoculation the tubes were incubated anaerobically. Illuminating gas (6) and hydrogen gas (7) anaerobiosis were both used in a few experiments but were found to be no more satisfactory than the simpler oat-jar method (8) in which standard anaerobe jars containing 3 inches of moistened oats at the bottom were sealed and evacuated by suction before being

[2] Two preparations of biotin were used: the methyl ester and the unesterified compound. No difference was observed in the activity of the two preparations.

[3] The medium containing ammonium phosphate (adjusted to pH 6.5) supported somewhat better growth and was, therefore, used in most of the final experiments.

placed in the incubator. This latter method was adopted for routine use, and all cultures were incubated at 37°C.

Supplements to basal medium: Crystalline biotin (or its crystalline methyl ester) was dissolved in basal medium and was added to culture tubes of the synthetic medium in appropriate amounts to make final concentrations of from 1.5×10^{-4} γ per cc. to 1.5×10^{-6} γ per cc. Basal medium containing p-aminobenzoic acid in final concentrations ranging from 1×10^{-3} γ per cc. to 1×10^{-6} γ per cc. was similarly prepared. The supplements were added to the basal medium prior to sterilization.

Estimation of bacterial growth: Cultures were examined for signs of bacterial growth either daily or at 12 hour intervals. Growth in the basal medium usually appeared first between 48 and 72 hours after inoculation. When gas production was noted in the tubes, the anaerobic jars were reevacuated by suction before being opened. Each tube was thoroughly shaken in order to obtain an even suspension of organisms. A 5 cc. sample of the culture was then pipetted into the chamber of the photoelectric colorimeter[4] and readings taken. Since the colorimeter readings vary inversely with the opacity of the solution tested, the readings recorded were inversely proportional to the amount of bacterial growth. When all of the tubes had been examined, they were replaced in the anaerobic jar which was sealed, reevacuated and returned to the incubator. The fact that 5 cc. of culture was needed for each colorimeter reading necessitated a relatively large volume (30 cc.) of medium in each culture tube at the start of an experiment. The identity of the organism cultured was frequently checked by smear and by plating on beef infusion agar. All growth curve experiments were run in duplicate or in triplicate.

RESULTS

The data summarized in Figure 1 illustrate the manner in which biotin, p-aminobenzoic acid, and both in combination affect the growth of *Cl. acetobutylicum* when added to the basal medium. The growth curves from typical experiments demonstrate that the addition of p-aminobenzoic acid and biotin in combination markedly stimulated the growth of both strains of *Cl. acetobutylicum*. The addition of biotin alone, on the other hand, was without effect, and p-aminobenzoic acid by itself caused only the slightest acceleration of growth. It should be pointed out that the growth-promoting effect of the latter compound may have been due to a synergistic action with traces of biotin carried over in the inoculum.

[4] A Leitz-Mass Clinical Photoelectric Colorimeter with a solid green glass filter (F–401) was employed in all experiments.

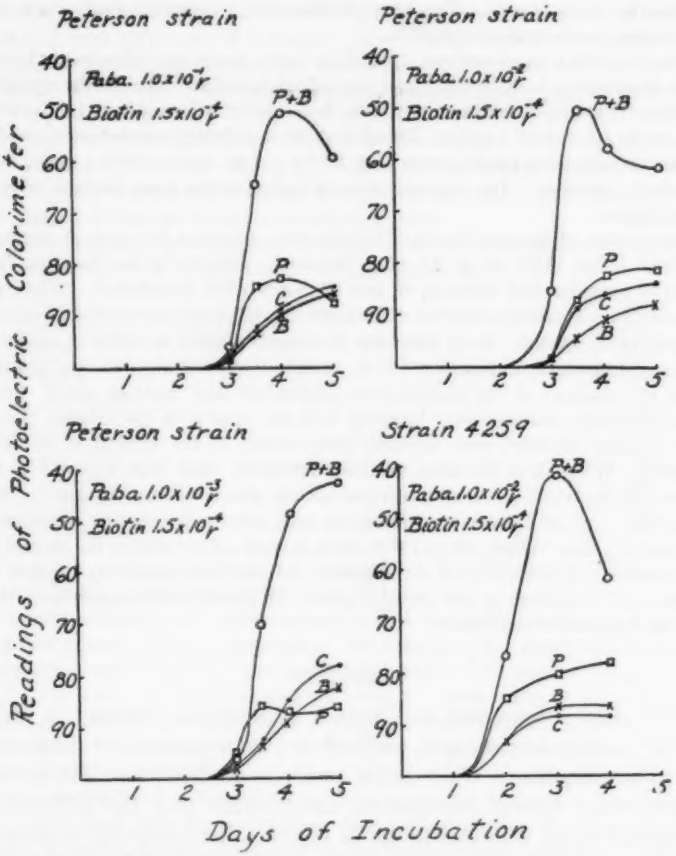

FIG. 1. THE GROWTH-PROMOTING ACTION OF p-AMINOBENZOIC ACID AND BIOTIN UPON CL. ACETOBUTYLICUM

Both strains of *Cl. acetobutylicum* grown in synthetic medium with growth factors added as indicated. Cultures incubated anaerobically at 37°C. Photoelectric colorimeter readings inversely proportional to density of medium; thus height of curves indicates extent of bacterial growth. p.a.b.a. = p-Aminobenzoic acid, B = Biotin, and C = unsupplemented basal medium.

In the presence of an excess of biotin[5] p-aminobenzoic acid was found to be extremely active as a bacterial growth factor. Experiments were carried out in which the concentration of biotin was maintained constant while that of p-aminobenzoic acid was varied from $1 \times 10^{-2}\gamma$ to $1 \times 10^{-6}\gamma$ per cc. The minimum concentration of p-aminobenzoic acid needed to support optimum growth was found to be $1 \times 10^{-6}\gamma$ per cc. At $1 \times 10^{-6}\gamma$ per cc. growth was definitely less than at higher concentrations. These results were obtained repeatedly with both the Peterson strain and the American Type Culture strain 4259. It is of interest that biotin is the only other known substance exhibiting biological activity at such infinitesimal concentrations.

Neither of the preceding sets of experiments conclusively demonstrates that biotin and p-aminobenzoic acid are the only supplementary factors needed for the growth of *Cl. acetobutylicum*. Since relatively large inocula were required to initiate growth, it is possible that other factors were carried over from the original peptone-containing medium. To eliminate this possibility cultures were passed serially in basal medium containing only p-aminobenzoic acid or p-aminobenzoic acid plus biotin. During five such serial passages there was luxuriant growth within 24 to 72 hours in all tubes containing both factors, but in those with only p-aminobenzoic acid no growth was observed after the third passage even though the tubes were incubated for more than a week.

DISCUSSION

These results suggest very strongly that p-aminobenzoic acid and biotin are the only supplementary growth factors required by *Cl. acetobutylicum* and they demonstrate conclusively that both factors (or their chemical equivalents) are needed to support the optimal growth of the organism. There is reason to believe that the "B Y" factor of yeast, shown by Oxford and his coworkers (4) to be a growth factor for *Cl. acetobutylicum*, serves as such only because it contains

[5] In combination with p-aminobenzoic acid, biotin was shown, during preliminary experiments, to cause maximum stimulation of growth at concentrations as low as 1.5×10^{-5} γ per cc. To insure an adequate supply of biotin in the medium, ten times this concentration (or 1.5×10^{-4} γ per cc.) was added routinely in subsequent experiments.

p-aminobenzoic acid or a closely related substance. p-Aminobenzoic acid is a known constituent of yeast (2, 9) and its chemical properties are such that it might be expected to have been extracted in the "B Y" fraction. Also, while the present study was in progress, Lampen and Peterson (10) independently demonstrated that p-aminobenzoic acid will replace the yeast factor, and concluded that the active substance in the yeast extract is p-aminobenzoic acid or some equivalent compound.

The experiments described above do not confirm the observation of Rubbo and Gillespie that p-aminobenzoic acid alone supports the growth of *Cl. acetobutylicum* in a similar synthetic medium.[6] No explanation can be offered for the findings of these authors, in the light of the present studies, unless possibly their basal medium contained traces of biotin. Although the experiments of Rubbo and Gillespie could not be directly confirmed, their final conclusion was amply substantiated, namely, that p-aminobenzoic acid is a bacterial growth factor for *Cl. acetobutylicum* and, therefore, according to the criteria enunciated by Fildes (11), is a substance probably concerned in the metabolism of other bacteria and one which may possibly play a role in cellular metabolism in general. The extraordinary biological activity of this compound as a bacterial growth factor suggests that it may be chemically related to a coenzyme.

SUMMARY

p-Aminobenzoic acid in the presence of biotin functions as a bacterial growth factor for *Cl. acetobutylicum* at concentrations as low as $1 \times 10^{-6}\gamma$ per cc. This finding suggests that p-aminobenzoic acid is a metabolite essential for bacterial growth and, therefore, may well be involved in the mechanism of action of the sulfonamide drugs.

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A STUDY OF MECHANICAL FACTORS IN THE CIRCULATION, WITH SPECIAL REFERENCE TO THE PROBLEM OF ACUTE CIRCULATORY FAILURE

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During a study of experimental shock in dogs the question frequently arose as to the importance of mechanical factors in acute circulatory depression and circulatory failure. Seeking an answer to the query, we constructed a mechanical circulation model with which we were able to carry out a series of studies analogous to those being made with dogs. The purpose of this paper is to present the results of those studies.

The dynamics of a circulation is obviously easier to investigate in a machine than in the body where variable factors are not readily controlled, measured, or evaluated, and where the picture is often confused by physiologic adaptations and pathologic changes. Although the most carefully designed model reproduces only very crudely the conditions which obtain in living vascular systems, and caution must be exercised not to press the analogy too far, the behavior of a properly constructed machine does offer valuable clues to the complex problem of hemodynamics.

Studies of a similar nature have been made by other investigators. Wiggers (1) used a circulation model of relatively simple design to study the effects of vasoconstriction and rigidity of arterial walls upon arterial pressure. In an investigation of the results of congestive heart failure, Starr and Rawson (2) employed a more elaborate apparatus with double circulation and with pumps which were believed to obey Starling's law of the heart. Bazett (3) has summarized studies made with a gravity-flow "schema" which represents only the arterial side of a single circulation,—studies which throw light on relationships of mean pressure to flow and resistance, of pulse pres-

sure to distensibility and stroke volume, and of diastolic pressure to pulse rate and systolic/diastolic ratio. We have designed a machine incorporating several important new features, and, as far as we are aware, most of the studies presented in this paper have not been made heretofore.

APPARATUS

Our circulation model is illustrated in Figures 1 and 2. It is a single circulation composed essentially of an actively pumping "ventricle," strongly elastic "arteries," "capillaries" which furnish variable resistance, a less strongly elastic and partially collapsible set of "veins," a passive "auricle," a "spleen," and accessory devices for taking measurements. A brief description of the various parts follows.

The *ventricle* is represented by a thin-walled rubber balloon inside a closed flask filled with water. Overdistention is prevented by the adjacent neck of the flask and a piece of silk netting. There are inlet and outlet valves. The balloon becomes in effect an active pump when small amounts of water are alternately forced into and sucked out of the flask through a tube which traverses and supports the ventricle but does not communicate with it. This movement of water is effected by means of positive and negative air pressure. The air-cocks, so arranged that when one is open the other is closed, are turned at the rate desired by an electric motor and sets of gears. In a single cycle, irrespective of rate, positive and negative valves are each open about 45 per cent of the time. By controlling the air pressures in the reservoirs, the pumping force of the ventricle can be regulated. Junction of air and water is in a calibrated glass tube where one may observe changes in the general level of the interface, indicating changes in ventricular volume. Cyclic excursion of the interface is a measure of ventricular stroke volume.

Arteries are represented by rubber tubes having medium-thick elastic walls. Glass distributing tubes are a little larger in inside diameter than adjacent rubber tubes. The "aorta" branches eventually into 128 small tubes which have a combined cross section area about 8 times that of the main vessel.

Capillaries are represented by 8 filters,—short brass cylinders, 2¼ inches in diameter, closed at the ends by rubber stoppers through which inlet and outlet tubes pass. These brass cylinders are tightly packed with fine-pore rubber sponges which are found to provide satisfactory and constant resistance Since this capillary system is enclosed within rigid walls, the volume of its fluid contents does not vary significantly. Rubber sponges such as we use, when completely filled with water, show negligible changes in volume (less than 2 per cent) when subjected to a pressure of 100 mm. Hg. Total cross section area of the capillary channels is estimated to be about 5 times that of the 128 smallest arteries, or 40 times that of the aorta.

In arrangement the *venous system* is the reverse of the arterial, 128 small tubes being tributary to a single "vena cava," which is represented by a large collapsible

Penrose tube. At corresponding levels, the cross section of the veins is about twice that of the arteries. The veins are not supplied with valves.

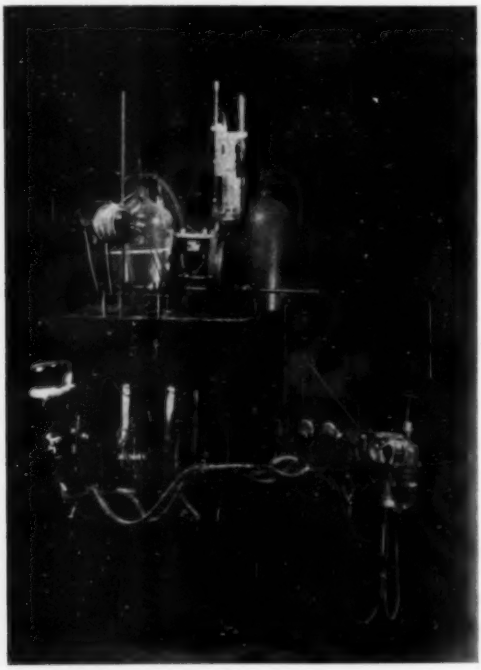

FIG. 1. PHOTOGRAPH OF THE CIRCULATION MODEL

The superstructure includes electric motor and gears, air pressure reservoirs, manometers, air-cocks, and air-pressure regulators. The circulatory system proper is on a lower level, being attached horizontally to the outsides of a box. From left to right may be seen: the ventricle in its flask, auricle, arteries, manometers, capillary filters and afferent tubes supported by a shelf, and spleen. Veins and flow meter are on the opposite side of the box and cannot be seen in this view. The tube connecting the air-cocks with the periventricular flask passes down through the box. For more detailed description see figure 2 and the text.

The *auricle* is represented by a thin-walled balloon nearly equal in size to the ventricle. An open glass capsule supports the auricle and prevents overdistention. The action of this auricle is passive.

The *spleen* is represented by a third rubber balloon fed by a small artery and drained by a large vein. The vein leads to the arterial end of a special low-resist-

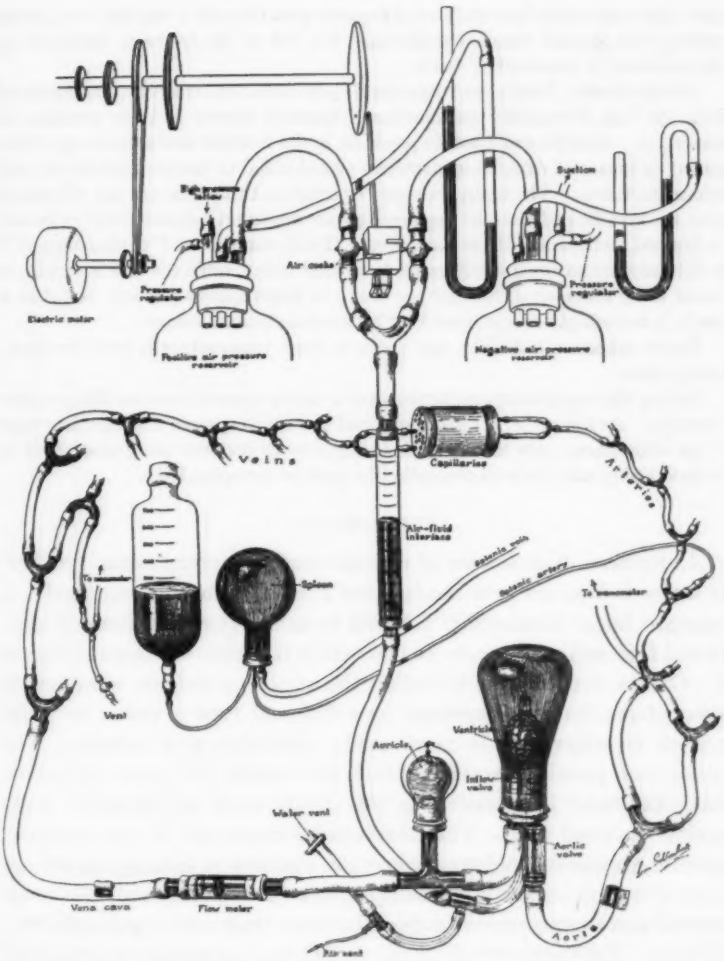

FIG. 2. A DIAGRAM WHICH SHOWS THE GENERAL PLAN OF THE MECHANICAL CIRCULATION MODEL

Only one of the eight capillary filters is drawn

ance filter (a "liver") which is not connected directly with the arterial system, but whose efferent tubes join those from other filters. That is to say, fluid enters the spleen under arterial pressure through a small vessel, and leaves through a larger

tube, but must under its own head of pressure pass through a capillary bed before entering the general venous circulation. The size of the spleen is measured by displacement of surrounding water.

Measurements: Positive and negative air pressures in the reservoirs are regulated with the help of mercury manometers. Accurate control of these pressures is important. Systolic and diastolic pressures in the ventricle and arteries are determined by means of damped manometers placed distal to sensitive maximum and minimum valves. The mean pressures reported in this paper are not calculated from the systolic and diastolic pressures but are measured independently by means of damped manometers at the heart level. Total volume flow ("cardiac output") is estimated by means of a calibrated flow meter which, when the flow is steady, is found to be accurate within ±2 per cent. A less accurate method, useful as a check, is to multiply rate of heart beat by measured stroke volume.

Unless otherwise indicated, tap water at room temperature is used for circulating fluid.

During the experiments particular care is taken to avoid suction-filling of the ventricle. As Figures 4 to 7 show, minimal ventricular pressure is kept near that of the atmosphere, only that amount of negative air pressure being used which is needed during each cycle to neutralize the positive pumping force.

EXPERIMENTS

A. Relation of distention of vascular walls to intravascular pressure. If water is injected into the isolated arterial system of our model, a constant linear relationship is found to exist between volume of contained fluid and pressure developed within the tubes as shown in Figure 3. On the venous side, including the auricle, a definite relationship also obtains, but it is expressed by a different type of curve since the system is larger and is more easily distended and collapsed. In either case positive pressure within the vessels (in excess of hydrostatic pressure) is produced by the elastic recoil of distended walls against enclosed fluid. The relationships expressed by the curves in figure 3 appear to hold true when the machine is running as well as when it is static; hence it is possible at any time to calculate from mean arterial and venous pressures the volumes of fluid contained in the two systems. Furthermore, since our model has a capillary reservoir of fixed capacity, and since the volume of ventricle and spleen can be measured independently, the distribution of fluid in the whole system can be readily calculated. The curves are modified, of course, by structural alterations in length, caliber, or elasticity of the tubes.

Somewhat similar relationships between volume and pressure prob-

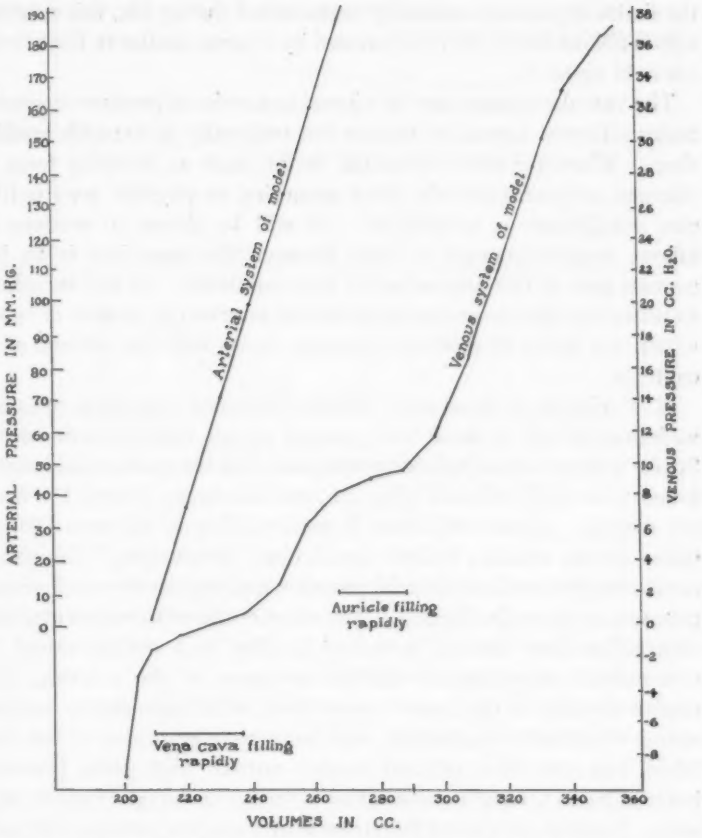
ARTERIAL PRESSURE IN MM. HG.
VENOUS PRESSURE IN CC. H_2O.
VOLUMES IN CC.
Arterial system of model
Venous system of model
Auricle filling rapidly
Vena cava filling rapidly

FIG. 3. RELATION OF VOLUME TO PRESSURE IN THE ARTERIAL AND VENOUS SYSTEMS OF THE MODEL

The plateaus in the venous curve correspond to periods when the collapsed, thin-walled vena cava and auricle fill with fluid, processes which require very little pressure. Since the vena cava and auricle are placed at different levels in relation to the ventricle, the former becomes distended before the latter begins to fill.

ably exist in the living circulation. Hollock and Benson (5) have determined the volume/pressure ratio for isolated segments of the human aorta shortly after death, and find that in young persons, within

the limits of pressure ordinarily encountered during life, this relationship is almost linear, and is expressed by a curve similar to the arterial curve in figure 3.

The vascular system may be viewed as a series of pressure reservoirs under different degrees of tension but ordinarily in dynamic equilibrium. Whenever some influential factor, such as pumping force, is changed, adjustments take place according to physical laws until a new equilibrium is established. As will be shown in sections to follow, reapportionment of fluid between the reservoirs is an important part of this adaptation to new conditions. It will be helpful to remember that in an elastic distended reservoir or system of tubes, within the limits of elasticity, pressure varies with the volume of its contents.

B. Variations in heart rate. Other conditions remaining constant, variations in rate of heart beat produce certain characteristic effects. Stroke volume varies indirectly with rate, but the mechanical heart is found to be most efficient when its rate is between 70 and 150 beats per minute. Above 150, there is poorer filling of the ventricle, reduced stroke volume, weaker ventricular "contraction," diminished cardiac output, reduced arterial pressure, and slightly elevated venous pressure, as shown in Figure 4. The effect of the weakened ventricular contraction (low maximal pressure) is offset to a certain extent by concomitant reduction of diastolic pressure in the arteries. Excessive slowing of the heart is associated with large stroke volume, strong ventricular contraction, and incomplete emptying of the ventricle, but also with reduced cardiac output, high pulse pressure, lowered mean arterial pressure, and virtually unchanged venous pressure. Shifts of circulating fluid from ventricular and arterial reservoirs to the veins, though small, are of fundamental importance in bringing about these changes. Increasing the volume of the venous reservoir by substituting a larger vena cava or auricle tends to negative the effects of bradycardia; or rather, the efficiency of the heart under such condition is not lessened until the rate falls to 40 or less.

Similar effects have been postulated or observed in experimental animals and in man. Grollman (6) says: ". . . moderate changes in pulse rate in normal individuals are without significant effects on the cardiac output. . . . There exists theoretically an optimal rate for the

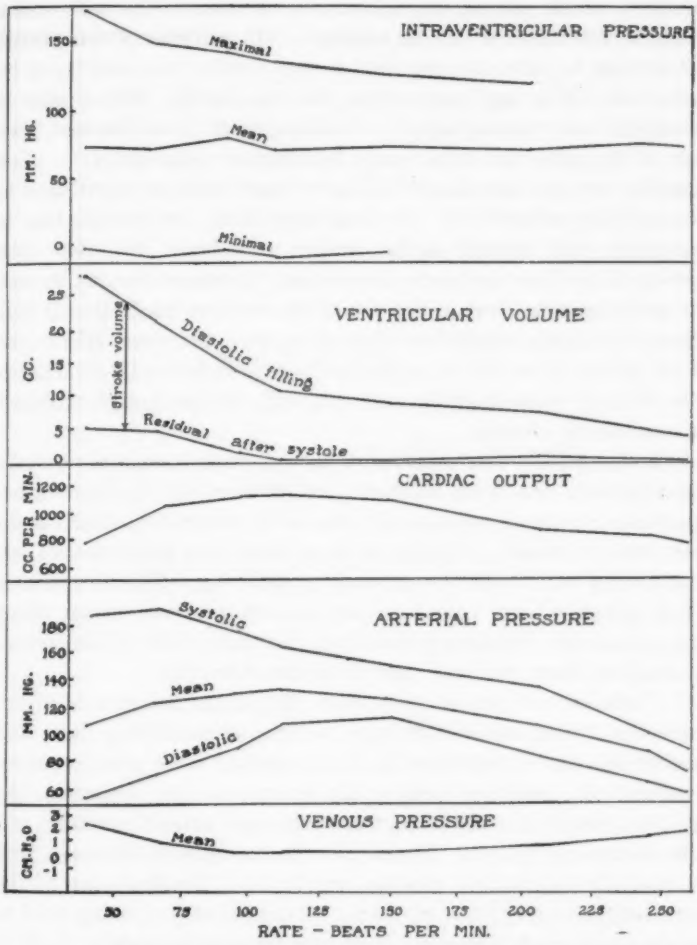

FIG. 4. MECHANICAL EFFECTS DUE TO VARIATIONS IN HEART RATE

The lag in our measuring device prevents accurate measurement of maximal and minimal intraventricular pressures when the machine is running rapidly. In this experiment the artificial spleen is excluded.

maximal cardiac output, any increase or decrease of this rate leading to slight diminution of cardiac output. . . . In paroxysmal tachycardia the increase in pulse rate may lead to such marked abnormality of the ventricular filling and contraction that the cardiac output may be diminished very considerably." Cardiac output is not lowered, however, if the pulse rate is increased by atropine (Marshall (7)). Low diastolic pressures associated with slow heart rates are mentioned by Boas and Goldschmidt (8). In total heart block, bradycardia may be associated with normal cardiac output (Grollman (6)), but acute slowing of the heart by vagus stimulation "decreases the cardiac output and allows the level in the venous reservoir to rise" (Bazett (4)). Figure 4 probably represents fairly accurately, therefore, effects produced in animals as well as in the machine by variations in heart rate. If so, it is not without significance that such changes can be explained on mechanical grounds.

Although positive air pressure in the reservoir (maximal potential pumping force) was kept constantly at 180 mm. Hg. throughout our experiment, maximal pressure recorded in the ventricle declined as the heart rate increased, probably because there was progressively less time during each cycle for pressure to build up. This decrease in actual pumping force coincides with reduction in ventricular filling during diastole. It appears, therefore, that under these circumstances the machine obeys Starling's law of the heart *in effect.*

C. Variations in force of heart beat. When the machine is so adjusted that the factors of heart rate, volume of circulating fluid, and number and size of capillaries are kept constant while pumping force is reduced (by lessening positive air pressure in the reservoir), the result is a progressive fall in cardiac output and arterial pressure, and a rise in venous pressure (Figure 5). Stroke volume decreases, and the ventricle empties less and less completely. Starling's law of the heart does not apply here, of course. Transfer of circulating fluid to venous reservoirs is striking. With rise in venous pressure there is increasing distension of thin-walled veins.

These changes are in certain respects like those observed clinically in cases of myocardial failure. It is important to note that in the model at any rate these adjustments are automatic and mechanical and take place when peripheral resistance remains apparently unchanged and when heart valves are unimpaired.

D. Variations in volume of circulating fluid. When potential pumping force (positive and negative air pressures), heart rate, and

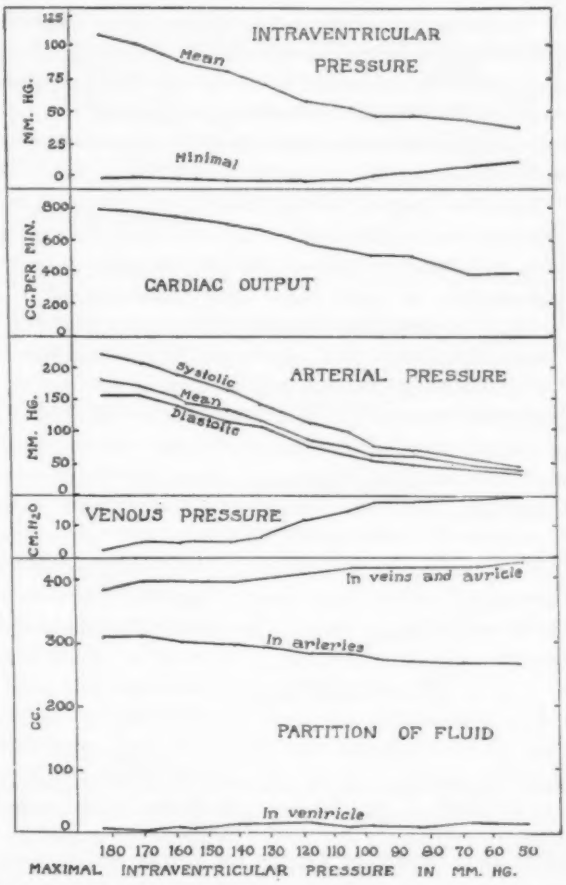

FIG. 5. MECHANICAL EFFECTS DUE TO PROGRESSIVE WEAKENING OF VENTRICULAR "CONTRACTIONS"
The artificial spleen is excluded

peripheral resistance are kept constant, gradual removal of fluid from our model produces a characteristic set of changes shown in Figure 6.

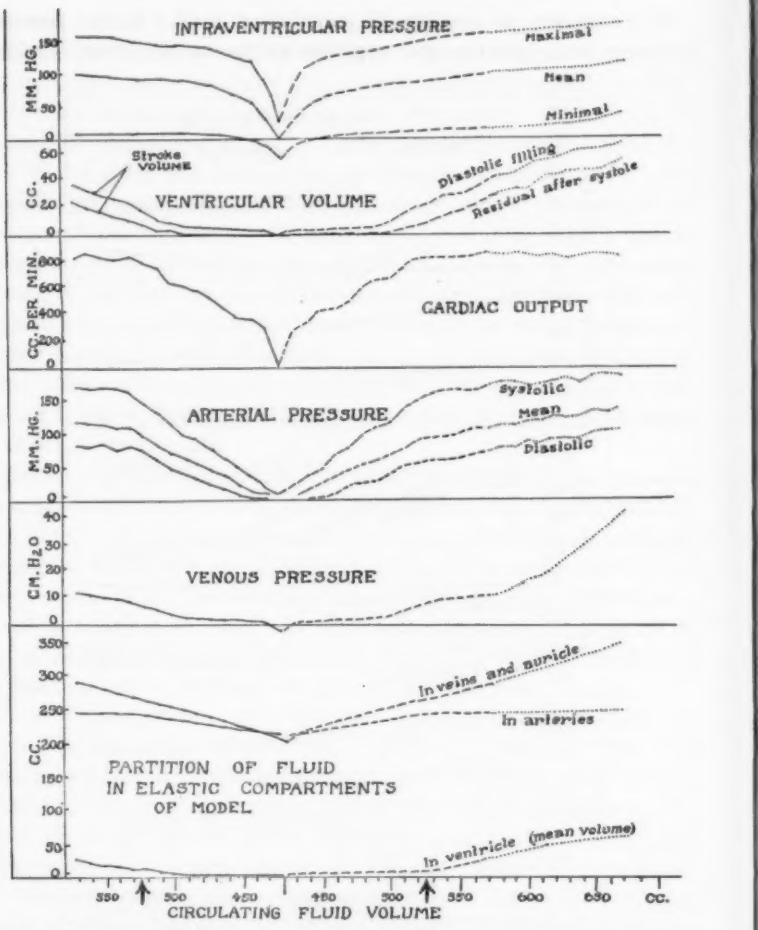

FIG. 6. MECHANICAL EFFECTS OF VARYING THE VOLUME OF CIRCULATING FLUID

Solid lines indicate results of the removal of fluid, broken lines the reinjection of fluid, and dotted lines the infusion of excess fluid. The artificial spleen is excluded. The minimum effective volume of circulating fluid (see text) is indicated by arrows.

Intraventricular systolic pressure, or force of contraction, varies roughly in proportion to ventricular filling during diastole, obeying in effect Starling's law of the heart. Arterial pressure declines slowly

at first, then more rapidly. Venous pressure, on the contrary, falls rapidly to begin with, then more gradually. Cardiac output is virtually unaffected by fluid loss at the outset, but soon after arterial pressure begins to fall, cardiac output decreases rapidly.

These effects are better understood if pressures are translated into volumes by means of the curves of Figure 3. Removal of fluid from the model,—it is immaterial whether fluid is taken from artery, capillary bed, or vein,—has the effect first of depleting the venous reservoir. This is the period of rapidly falling venous pressure, and of sustained arterial pressure and cardiac output,—a period the length of which depends upon the functioning capacity and initial distention of the venous compartment and accessory reservoirs, such as the spleen. It is only after large veins begin to collapse and central venous pressure is low that the ventricle fills poorly, stroke volume is reduced, cardiac output decreases, and arterial pressure declines rapidly as volume of fluid in the arterial compartment is reduced. The two systems, arterial and venous, respond differently because they are not in *free* communication, and because the one is a high-pressure reservoir with elastic, distended walls, whereas the other is a low-pressure reservoir whose walls are less elastic and more collapsible.

It should be noted that as the volume of circulating fluid in the machine decreases, arterial pressure and cardiac output become increasingly sensitive to fluid loss. Figure 6 shows that at the outset, removal of 10 cc., or even 50 cc., has relatively little effect, whereas later a 10 cc. loss depresses the circulation severely.

Reinjection of fluid reverses the curves. The auricle and ventricle, which have been emptied by fluid loss, are distended by the infusion. Holman (9) has shown by roentgenographic studies that in dogs also an immediate effect of bleeding and refusion of blood is to alter the size of the heart. Overdistention of this artificial circulation, something not easily effected in experimental animals, raises both arterial and venous pressures. If these pressures are raised by equal amounts by the infusion, as in Figure 6, the pressure differential which controls capillary flow remains unchanged, and consequently cardiac output is unaffected. When somewhat more rigid tubing is used for arteries, overdistention of the system causes arterial pressure to rise more rapidly than venous, and consequently there is an increase in volume

flow. With less elastic veins, on the other hand, injection of excess fluid has the effect of reducing the cardiac output.

There is a remarkable similarity, particularly in the form of the curves, between the effects just described and those which result from hemorrhage in animals (10). Certain differences should be pointed out, however. The animal shows a tendency toward spontaneous recovery, which of course is absent in the model. On the other hand, replacement of blood often fails to restore an animal fully to normal, especially if the transfusion is delayed for a time, whereas in the machine reinjection of fluid restores all the original conditions. The inference to be drawn is that the effects of uncomplicated acute hemorrhage upon the animal circulation are fundamentally hemodynamic, even as regards the major characteristics of the blood pressure and cardiac output curves, but that these effects may be modified to a certain extent by physiologic adaptations and pathologic changes.

This experiment not only implies that there may be mechanical reasons why considerable amounts of blood may be lost without much effect upon blood pressure, but it also offers an explanation of why individuals differ so greatly in ability to withstand severe bleeding. It suggests, moreover, why small hemorrhages may cause serious or fatal reactions in an oligemic individual, and why relatively small transfusions are often surprisingly effective in raising blood pressure and improving blood flow in states of posthemorrhagic hypotension.

To explain circulatory depression in shock many investigators have advanced the idea of a disparity between blood volume and vascular capacity. It is a concept which in our opinion does not bear analysis. An open rigid container like a flask, to be sure, has a specific capacity, and when such a container is partly filled with fluid there is obviously a disagreement between the volume of the liquid and the capacity of the flask. This comparison becomes less meaningful, however, if the flask is sealed and contains only fluid (i.e., no free gas), for in that case the volume of fluid, the internal volume of the flask, and the capacity of the flask are necessarily equal. If, instead of a flask, we have an elastic rubber balloon containing water and no free gas, the internal volume of the receptacle might vary tremendously depending on the volume of water, but its capacity would be an ill-defined, uncertain

matter. In such a case it would seem pointless to speak of a disparity between fluid volume and balloon capacity. The cardiovascular system is a closed reservoir, containing variable but measurable amounts of blood and no free gas. Its walls are collapsible and distensible. Its internal volume is always equal to the volume of blood, —otherwise a vacuum would be created. Its capacity, however, is an

TABLE I

Approximate Volume of Fluid in the Elastic and Collapsible Parts of Our Model under Various Conditions*

	MACHINE AT REST; "EXSANGUINATED;" VENOUS PRESSURE −12 CM. H_2O.	MACHINE RUNNING; ORDINARY CONDITIONS OF USE; VENOUS PRESSURE +10 CM. H_2O.	MACHINE AT REST; SYSTEM EXCESSIVELY DISTENDED; VENOUS PRESSURE +35 CM. H_2O.
	cc.	*cc.*	*cc.*
Heart:			
Ventricle	0	20	85
Auricle	0	20	70
Arteries	210	250	270
Veins:			
Thicked-walled tubes	200	210	220
Thin-walled tubes	0	70	110
Spleen	0	150	500
Totals	410	720	1255

* This table does not include the fluid in connecting glass tubes and other rigid parts of the machine. That fluid volume, which is estimated at 685 cc. (50 cc. in the heart valves, 60 cc. in arterial connecting tubes, 470 cc. in the capillary system, 105 cc. in venous connecting tubes and flow meter) is invariable, and is therefore properly disregarded when considering relations of volume, distention, and pressure. This has been done in figures 3, 5, 6, 7, and 8, and in Table II.

indefinite, variable, and perhaps indeterminable quantity; degree of filling (in relation to capacity) is not susceptible of measurement; and "adequate" filling can be defined only in terms of circulatory function, —the very thing the investigators set out to explain.

Table I, which shows the approximate volume of circulating fluid in our model under various conditions, illustrates the difficulty of determining vascular capacity and degree of filling, even in a machine.

E. Variations in peripheral resistance. Most of the tests described so far were made with 3 of our 8 filters clamped off on the arterial side, though they remained open to the venous circulation. By shutting off more or fewer filters it was possible to vary peripheral resistance while other factors, such as heart rate, air pressure, and fluid volume, were kept constant.

The complex effects of such variations are most clearly expressed in a diagram (Figure 7a). It will be seen that increasing the resistance by using less than 5 filters causes arterial pressure to rise moderately, cardiac output to fall, venous pressure to decline sharply toward zero, and pulse pressure to decrease both absolutely and relatively. These changes are associated with redistribution of circulating fluid, the venous compartment losing water to the arterial compartment, and especially to the ventricle, which becomes distended and empties inefficiently. There is a concomitant rise in intraventricular pressure.

Lessening the peripheral resistance, on the other hand, by opening filters 6 and 7, causes a distinct fall in arterial pressure. Pulse pressure is increased absolutely and relatively. Cardiac output and venous pressure are but slightly changed, however. Fluid is shifted from the arterial to the venous side.

When this experiment is repeated with considerably less fluid in the machine, and consequently with reduced initial pressures and cardiac output, the effects produced by changes in peripheral resistance are similar to those just described, except that distention of the ventricles is less pronounced or is absent.

This method of varying peripheral resistance has its counterpart in the living circulation when certain regions are temporarily closed to the arterial circulation.

Since with our machine one may start with 6 filters and cut off one of them without appreciably reducing the total volume flow (Figure 7a), it appears that under certain conditions decreasing the number of capillary channels may be compensated for by an increased rate and volume of flow through each of the remaining capillaries, even though these functioning channels have not changed in size of lumen. The explanation probably lies in the rise of arterial pressure. In experimental shock selective vasoconstriction causing reduced blood flow through the extremities and certain other regions may be associated with relatively well maintained flow through vital organs. It is a

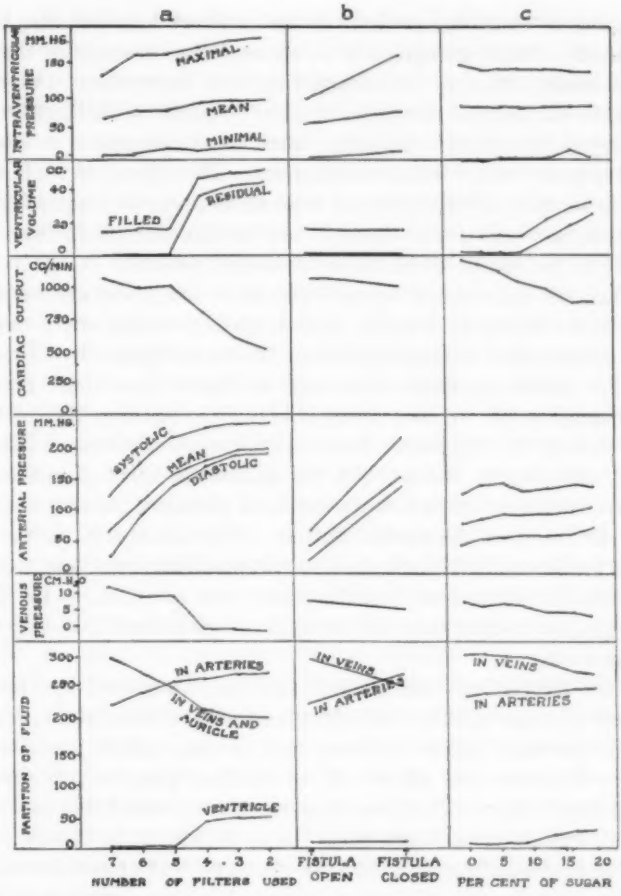

FIG. 7. MECHANICAL EFFECTS OF INCREASING PERIPHERAL RESISTANCE: (*a*) BY REDUCING THE NUMBER OF ACTIVE CAPILLARY CHANNELS, (*b*) BY CLOSING AN ARTERIOVENOUS FISTULA, AND (*c*) BY INCREASING THE VISCOSITY OF THE CIRCULATING FLUID

phenomenon which occurs even in the absence of any rise in arterial pressure, suggesting the influence of an additional factor such as reciprocal vasodilatation.

In animals, peripheral resistance is ordinarily varied not only by

including or excluding sections of the capillary bed, but also by the constantly changing degrees of vasoconstriction throughout the body which modify the flow without cutting it off altogether. In our machine we can imitate this effect crudely by partly occluding the tubes leading to the filters. Actually, these tubes correspond to medium sized arteries rather than to arterioles. The effects of such a procedure are only slightly different from those shown in Figure 7a; pulse pressure decreases more sharply, and cardiac output declines more nearly in proportion to elevation of arterial pressure.

When the arterial and venous systems of our model are connected directly by means of a $\frac{1}{4}$-inch tube, a systolic venous wave appears, and certan other changes which are shown in Figure 7b. These effects, it should be noted, differ only in degree from those obtained by increasing the number of capillaries. On opening the "fistula," flow through the capillaries is no doubt greatly diminished. In animals, according to Holman (9), the immediate effect of establishing an arteriovenous fistula is to lower blood pressure, increase the heart rate, and increase the cardiac output. This increase in cardiac output is attributed by Holman to acceleration of the heart beat. In our machine, cardiac output is not increased very much unless the fistula is large, and in that case the increase occurs irrespective of changes in heart rate.

If the circulating water in our machine is replaced by an equal volume of sugar solution, arterial pressure is raised slightly, whereas venous pressure, stroke volume, and cardiac output are reduced. Figure 7c shows the effects of substituting progressively stronger solutions of sugar. This procedure represents a condition sometimes seen in experimental shock where there is little or no change in the volume of circulating blood but the viscosity of the blood is increased due to a rise in the cell/plasma ratio. We have found that under such conditions dogs have comparatively high blood pressure associated with low cardiac output.

These experiments illustrate what we believe to be a principle in hemodynamics,—that changes in peripheral resistance tend to have opposite effects upon arterial pressure and cardiac output. Many phenomena observed in experiments on animals fall in line with this general rule. In the living circulation, closure of an arteriovenous

fistula, producing a sudden increase in peripheral resistance, raises arterial pressure and reduces the output of the heart. In certain types of experimental shock, characterized by hemoconcentration and widespread vasoconstriction, we have found that a deceptively high blood pressure may be associated with a dangerously inadequate blood flow; and in another type of shock, in which there is generalized vasodilatation, blood pressure may be greatly reduced with surprisingly slight injury to the animal because the cardiac output is well sustained. It has been thought by some that increased peripheral resistance actually causes increased cardiac output because of application of Starling's law of the heart.[1] One should differentiate here between mechanical effects and the modifying influences of physiologic activity which sometimes result in overcompensation. In common with other investigators (14), we have found that small doses of adrenalin may raise both blood pressure and cardiac output, whereas large doses greatly elevate blood pressure but reduce the minute volume output. Barcroft (15) has brought forward evidence to show that the influence of adrenalin upon the peripheral resistance tends to decrease systemic blood flow; that the influence of adrenalin upon the cardiopulmonary system tends to increase the systemic flow; and that the variable results of adrenalin injections appear to be due to variations in the extent of its influence upon the peripheral resistance and upon the cardiopulmonary system. Furthermore, we have increased and decreased experimentally the viscosity of circulating blood in dogs, and thus have varied the peripheral resistance, by injecting or removing blood cells, taking care meanwhile not to alter the blood volume significantly. The results of these experiments lend further support to our contention that changes in peripheral resistance tend to have opposite effects upon arterial pressure and cardiac output.

F. Modifying effects of a depot of fluid. To represent the spleen

[1] Reference is often made to the original work of Starling and his associates (12, 13) with heart-lung preparations. Those studies are not relevant to the present consideration, however, since a condition of the experiments was arbitrary regulation of cardiac inflow from a reservoir. They show that, given constant inflow and heart rate, increase in arterial resistance causes distension and incomplete emptying of the ventricle and elevation of arterial pressure.

we used an apparatus which has some of the fundamental features of a blood depot,—a distensible reservoir with low elastic constant, an arterial inlet smaller than the venous outlet, and a capillary bed interposed between the splenic vein and the general venous circulation.

We find that such a depot tends automatically to counteract the effects of removing fluid from the circulation, or of adding fluid to it, and that cardiac output and arterial pressure remain virtually unaffected by fluid loss until the spleen has discharged a large portion of its contents. It appears that circulating fluid taken from a vein is replaced almost quantitatively by splenic fluid, and as long as that replacement continues the volume of fluid in the arterial and venous systems remains nearly constant. Conversely, excess fluid injected into a vein is stored away quantitatively in the spleen until the spleen approaches the limits of its distensibility. This compensatory action is brought out in Figure 8 by a comparison of the two curves of mean arterial pressure, the solid line representing pressure when the spleen is in use, and the dotted line the effects of repeating the experiment with splenic artery and vein clamped off. The presence of such a depot of fluid in the model modifies only slightly, however, the characteristic responses of the circulation to variations in heart rate, pumping force, and peripheral resistance.

Obviously, the size of this mechanical reservoir is determined by the relative rates of inflow and outflow, which in turn are dependent upon pressure differentials between arteries and spleen, and between spleen and veins. Since a small fluid loss affects venous pressure more than arterial, splenic inflow tends to remain unchanged whereas outflow is increased, and this discrepancy continues until a new equilibrium is reached with venous volume and pressure largely restored, with arterial pressure unaltered, and with splenic volume stabilized at a low level. Rapid removal of fluid may cause a sharp fall in both cardiac output and arterial pressure; but as the spleen empties, these curves slowly return to their original levels, and the final result is the same as though the fluid had been removed slowly. When resistance in the "liver" of our model is increased, the spleen becomes distended and the volume of the more actively circulating fluid is reduced correspondingly. Contraction of the spleen, simulated by

forcing water into the perisplenic flask, has the effect of increasing the volume of fluid in the arterial and venous systems.

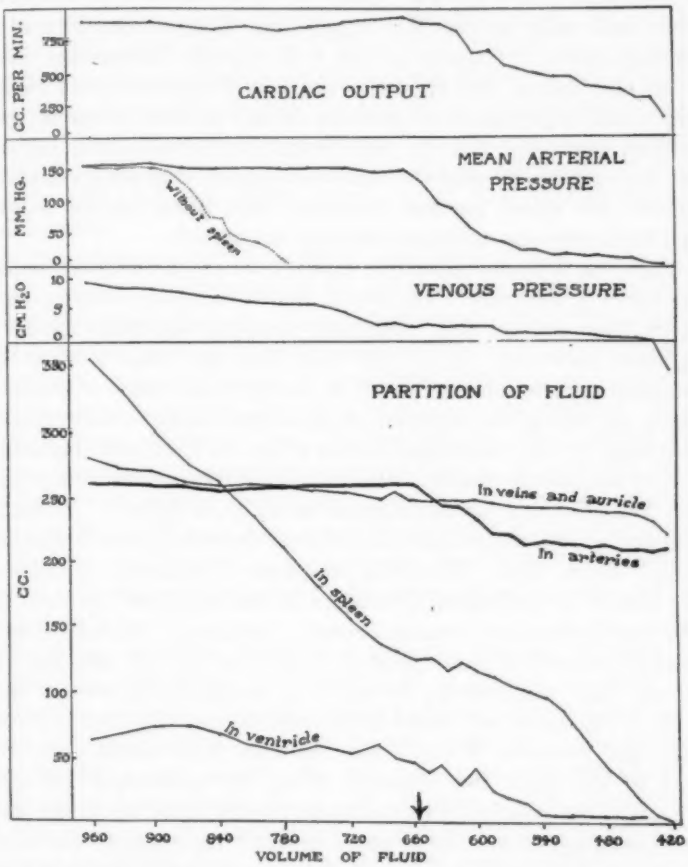

FIG. 8. MECHANICAL EFFECTS OF REDUCING THE VOLUME OF CIRCULATING FLUID IN THE PRESENCE OF A LARGE, WELL-FILLED FLUID DEPOT
Minimum effective volume is indicated by the arrow

The mechanism which regulates blood flow and blood storage in the living spleen has never been fully understood. Some investi-

gators have postulated sphincter-like control of afferent and efferent vessels, or highly specialized connections between arterioles and venous sinuses and between sinuses and pulp spaces. The observations made with our machine suggest that filling or emptying of an animal's spleen in response to rest, food, emotion, hemorrhage, adrenalin, barbiturates, and like influences, may be in part at least simply mechanical adjustments to extrinsic factors of blood pressure, generalized vasomotor activity, and contraction of the splenic capsule. We have repeatedly observed that when normal dogs are given transfusions, the spleen becomes distended with blood, cardiac output and blood pressure remaining virtually unchanged.

Even less is known about the existence or control of blood depots elsewhere in the body. The portal system of veins, including those in the liver, and the large pulmonary veins are sometimes spoken of as blood reservoirs. If it is true that these and other parts of the vascular system serve at times as inactive reservoirs of stagnant blood, the filling and emptying of such depots might conceivably be controlled by the mechanical factors which we have been discussing.

G. Variations in posture. Most of our experiments were made with the circulation in a horizontal plane, as shown in figure 1. In such a position hydrostatic pressure in the most dependent vessels does not exceed 20 cm. H_2O. When this arrangement is altered by changing the level of the peripheral circulation in relation to the ventricle, the effects of hydrostatic pressure become pronounced. If, for example, arterial and venous pressures at the heart level are 125 mm. Hg. and 10 cm. H_2O respectively, pressures in corresponding vessels on a level 30 cm. higher are found to be only 103 mm. Hg. and -20 cm. H_2O, and pressures 30 cm. below the heart level are 147 mm. Hg. and 40 cm. H_2O, the difference being the hydrostatic pressure. Changing the position of our artificial capillaries as far as the slack in afferent and efferent tubes would permit (from about 40 cm. above to 40 cm. below the heart level) did not alter the cardiac output; however, nor were the arterial and venous pressures at the ventricular level affected to any extent. And theoretically, the velocity flow through our inelastic capillary filters was not altered by changes in posture because the arteriovenous pressure differential remained the same.

We repeated all the foregoing experiments (variations in heart rate, force of beat, volume of fluid, etc.) with our model in non-horizontal positions. The results were similar to those already described, provided we kept all easily distensible portions of the circulation (spleen, vena, cava, auricle) on a level with the ventricle. In these experiments the mechanical circulation was unaffected by changes in position presumably because the amounts of hydrostatic pressure produced were not sufficient to constrict or dilate the thick-walled rubber tubes appreciably, and consequently the movement of fluid was controlled by laws which govern rate of flow through siphons and U-tubes.

In the animal circulation, however, the effects of these mechanical forces may be greatly modified by distension or collapse of blood vessels and capillaries from hydrostatic pressure. Only when a body is immersed in water, and intravascular hydrostatic pressure becomes neutralized, does regional and general blood flow show an independence of posture analogous to that evidenced by our machine. It is not surprising, therefore, that postural changes should affect the human circulation; the surprise, rather, is that the effect should be as little as it is in healthy individuals. This tendency toward stability is due in part to the mechanical factors illustrated by the behavior of our machine, and in part to such aids to the circulation as venous valves, and the pumping action of muscular movements and of respiration.

We performed a few experiments with our machine in which we lowered the thin-walled vena cava until it became distended by hydrostatic pressure. The effect was to depress the circulation very much as though a quantity of fluid has been removed. This observation, incidentally, may throw some light upon a phenomenon encountered in our animal experiments. As has been pointed out already (in section D), small bleedings have almost no effect upon the blood pressure of normal dogs, whereas in severe posthemorrhagic shock similar blood losses are apt to reduce the blood pressure considerably. In like manner, changing the position of anesthetized, otherwise normal dogs is found to have little effect upon blood pressure, whereas lifting one end of a shocked, relaxed, oligemic dog may cause a sharp, or even fatal, fall in arterial pressure.

H. Compensatory effects in circulatory depression. As has been shown, the minute-volume flow in our model can be reduced by feeble ventricular contraction, excessive slowing or acceleration of heart beat, reduce fluid volume, increased viscosity of circulating fluid, vasoconstriction, or closing off of a large portion of the capillary bed. In every case an adaptation to new conditions occurs which consists essentially of an automatic shift of fluid from a depot to the active circulation, or from one compartment to another. There are other adjustments, however, which can be made by the operator,—adjustments which correspond to physiologic adaptations in the living body. It is important to determine what compensatory effects these can have. If the artificial circulation is impaired by fluid loss, for example, can other factors be so adjusted as to restore the circulation to "normal," or is it necessary to replace the lost fluid?

(a) When cardiac output is seriously reduced by enfeeblement of ventricular contraction, slight improvement results from increase of fluid volume; but modification of the other factors has no compensatory value.

(b) The reduced minute-volume flow which results from slowing the heart rate to 40 or 30 beats per minute (Figure 4) can be corrected by increasing the volume of actively circulating fluid or by increasing the force of ventricular contractions. In excessive tachycardia the circulation is somewhat benefited by increasing the force of ventricular contraction, but not by any other adjustments that we were able to make. In either case, raising peripheral resistance causes elevation of mean arterial pressure but a further decline in cardiac output.

(c) When the circulation is depressed by fluid loss, no benefit derives from increase in heart rate alone, although improvement follows strengthening of the ventricular contraction, or a combination of the two. Further improvement is observed if the artificial ventricle is given a few millimeters of negative pressure during diastole, such as might well occur in the living circulation during forced inspiration. These compensatory effects are most striking when the cardiac output has been only slightly reduced by fluid loss; they become progressively less influential with the approach of circulatory failure from excessive fluid loss. Shutting off a small portion of the

capillary bed may raise arterial pressure without reducing the cardiac output (Figure 7), but a large increase in peripheral resistance elevates arterial pressure at the cost of cardiac output and volume flow generally.

(d) The slow circulation which results from increased viscosity of fluid can be overcome by strengthening the ventricular beat, or by using more or larger capillary channels.

(e) In our machine reduced volume flow associated with arteriolar (arterial) constriction or cutting off of part of the capillary bed can be improved only by procedures which at the same time greatly elevate the arterial pressure. Theoretically, a corrective would be to use a circulating fluid having lower viscosity.

COMMENT

Other factors remaining constant, a limited reduction of the volume of fluid in the model produces a condition characterized by a poorly filled venous system and spleen and low venous pressure, associated with "normal" cardiac output and well sustained arterial pressure. This *minimum effective volume* is shown in figures 6 and 8. If fluid loss is carried further, cardiac output and arterial pressure fall rapidly. If, on the other hand, fluid is reinjected to raise the volume above the amount necessary for efficient circulation most of the excess fluid is received into the venous reservoir or is stored in the spleen, and hence the cardiac output tends to remain unaltered and arterial pressure is increased only slightly. It may be said, therefore, that as long as the total quantity of fluid remains above the minimum effective volume neither removal nor addition of fluid has much effect upon volume flow through heart and capillaries, but when the model contains less than the minimal effective volume of fluid, any change in fluid contents alters the rate of flow.

It is reasonable to suppose that an analogous situation obtains in the living circulation. Such an assumption would help to explain some of the results of experimental hemorrhage. For example: In 21 large healthy dogs we found that individual plasma volumes varied from 21 to 63 cc. (mean 51.1 cc., standard deviation 6.4 cc.) per kg. of body weight. We also found that some of the animals suffered comparatively large blood loss without significant change in blood

pressure and cardiac output, whereas others manifested depression of the circulation almost from the onset of bleeding. Furthermore, the amounts of blood which had to be removed to produce shock (persistent hypotension associated with anoxia) ranged from 20.2 to 58.9 cc. per kg. of body weight, but most of this variation occurred in the period before the blood pressure began to fall sharply, and there was less variation in the second period during which the blood pressure was falling rapidly to a shock level. Expressed statistically, the coefficient of variation in blood losses per kilogram during the first period was about twice that of the second period.

TABLE II

Percentages of Original Volumes of Circulation Fluid or Plasma Which Must Be Removed in Order to Produce Certain Degrees of Circulatory Depression*

	FROM BEGINNING OF EXPERIMENT TO MINIMUM EFFECTIVE VOLUME	FROM MINIMUM EFFECTIVE VOLUME TO 50 MM. MEAN ARTERIAL PRESSURE	FROM 50 MM. PRESSURE TO CESSATION OF CIRCULATION	TOTALS
Model:				
With spleen	39.5	12.1	24.2	75.8
Without spleen	37.3	13.0	9.2	59.5
Average of 20 dogs	34.7	20.3	2.6	57.6

* Since blood volume in the living circulation cannot in our opinion be measured with any certainty, these calculations are based upon intravascular plasma volume (determined by a dye-dilution method) and the plasma content of blood removed.

It is instructive also to compare the mechanical and animal circulations in regard to the proportions of total circulating fluid which must be removed in order to produce certain degrees of circulatory depression. In this study the machine contained sufficient fluid at the start to produce a venous pressure of +10 cm. H_2O. Fluid was then removed (a) until arterial pressure began to fall sharply, (b) until mean arterial pressure reached 50 mm. Hg., and (c) until circulation ceased. Similar observations were made in the dogs, none of which had been splenectomized. The results are summarized in Table II.

Although in animals physiologic and pathologic factors come into play, especially during the shock period (third column), the parallelism in this table is sufficiently close to emphasize the fundamental im-

portance of mechanical factors in posthemorrhagic hypotension and circulatory failure.

In a recent study of the effects of acute experimental hemorrhage (10) a question was raised as to the cause of terminal, sudden collapse of the circulation following periods of posthemorrhagic shock, since no immediate changes in blood volume, vasomotor activity, or capillary permeability could be found to account for it. A similar effect can be produced in our machine by suddenly reducing the positive air pressure (potential pumping force), in which case intraventricular pressures fall sharply toward zero earlier than in Figure 6. This observation suggests the possibility that weakening of ventricular contractions in late stages of shock may be a factor in bringing about the terminal "break" in blood pressure.

Finally, our model lends itself to a further consideration of peripheral resistance and velocity flow through the small tubes and capillaries. There is a constant relationship between cardiac output, peripheral resistance, and arterial pressure. As Wright (11) puts it: "The blood pressure varies as the product of the cardiac output and the peripheral resistance." Bazett (4), from studies made with a schema, reached similar conclusions, and showed that the pressure concerned is mean arterial pressure. Furthermore, frictional resistance encountered by fluids passing through narrow tubes depends on viscosity, size of lumen, and velocity of flow (Reynold's index law). If, therefore, viscosity of fluid and size of lumen are constant, as in most of our mechanical experiments, peripheral resistance will vary with velocity of flow.

Since arterial pressure and cardiac output are measurable, relative changes in peripheral resistance and velocity flow can be estimated. Review of our experiments from that standpoint brings out the following points:

Changes in rate of heart beat influence arterial pressure and cardiac output almost equally (Figure 4), from which it may be deduced that velocity flow through capillaries is not greatly altered by either bradycardia or tachycardia. Weakening of ventricular contraction, however, reduces arterial pressure proportionately more than it reduces cardiac output (Figure 5), indicating a lessened velocity flow. Likewise, when the volume of circulating fluid is decreased, arterial pres-

sure falls somewhat more rapidly than cardiac output (Figure 6), a relationship which shows that velocity flow in the small tubes and capillaries is diminished. When peripheral vessels are reduced in number (Figure 7a) or in size, peripheral resistance is of course increased, and this increase is reflected in the high arterial pressure and low cardiac output.

In animals, total peripheral resistance in the body can in like manner be estimated from cardiac output and mean blood pressure, but the three factors in resistance (viscosity, size of lumen, and mean velocity) are so variable, uncontrollable, and complex in their relationships as to defy accurate analysis. Certain general deductions are possible, however. In our study of experimental hemorrhage (10), for example, we did not find that blood pressure falls more rapidly than cardiac output, as might be expected from the behavior of the model (Figure 6), or as might be expected in view of the demonstrable reduction in blood viscosity from dilution. On the contrary, following hemorrhage cardiac output was observed to decrease somewhat more rapidly than mean arterial pressure, indicating a slight rise in total peripheral resistance. This rise must be attributed, therefore, to the remaining factor, vasoconstriction.

SUMMARY AND CONCLUSIONS

Many features of the normal blood circulation have been reproduced in a mechanical circulation model, and certain changes which characterize acute circulatory failure have been studied experimentally in the machine and in dogs. The behavior of the machine under controlled conditions provides a number of suggestive clues to the complex problem of hemodynamics. The general impression received by the authors is that the living circulation is influenced by mechanical factors to a degree not generally appreciated heretofore.

The mechanical circulation is essentially a series of fluid-filled, pressure reservoirs in dynamic equilibrium. When this balance is upset by external factors, the machine tends automatically to establish a new equilibrium by redistributing its circulating fluid between the different vascular compartments. It is suggested that under analogous conditions similar adjustments take place in the animal.

On the basis of this study the following statements are believed to

express general principles in hemodynamics: blood pressure has a definite relationship to the elasticity and distension of the vascular system; changes in peripheral resistance tend to have opposite effects upon arterial pressure and cardiac output; effects of postural changes upon regional and general blood flow depend upon the degree of dilatation or collapse of blood vessels produced by the variations in hydrostatic pressure, as well as upon the efficiency of venous valves and the pumping action of respiration and other muscular movements; peripheral resistance varies with velocity of flow, viscosity of blood, and size of blood channels.

The view that acute circulatory failure, such as occurs in shock, may be due to a disparity between blood volume and vascular capacity is criticized.

The concept of a minimum effective blood volume is introduced, and reasons are suggested why comparable individuals vary so greatly in blood volume, in susceptibility to blood loss, and in response to blood transfusion.

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THE REACTION OF THE SUBCUTANEOUS TISSUES TO THE ACETONE INSOLUBLE LIPOIDS FROM BEEF BRAINS

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In contrast to the formation of epithelioid cells and fibrous tissue following subcutaneous injection of various other substances, two commercial preparations of lecithin were found to stimulate infiltrations of macrophages of exceptional size and phagocytic activity which disappeared without transformation into epithelioid cells, and without fibrosis (35). These commercial products were the egg lecithin manufactured by the Digestive Ferments Company, and an emulsion of brain lipoids called F.L.2 which was extracted by the Wilson Laboratories. The reaction to the latter was the more striking. Brain lipoids were therefore used for the subsequent studies directed toward an explanation of the findings. These studies have been presented in abstract (36) and form the basis of this report.

MATERIALS AND METHODS

The lipoids used for these studies were obtained from three sources. First, Dr. David Klein of the Wilson Laboratories kindly donated a supply of the dried brain lipoids from which the F.L.2 had been prepared. This dried material consisted of the acetone insoluble lipoids in a benzol extract of beef brains which had been previously extracted with acetone. It consisted largely of ether soluble lipoids but proved to contain also a small amount of ether insoluble ones, which were, however, insufficient for further needs. Therefore, fresh calf and beef brains were used as the other two sources, respectively. The lipoids from each of two calf brains, and two beef brains, were subjected to individual extraction and experimental biologic assay. Those from the calf brains contained relatively less of the ether insoluble fractions than did those from the beef brains.

I. Extractions. The extractions of the various lipoidal fractions were performed by the methods of differential solubility discussed in detail by MacLean and MacLean (26). The details are, therefore, omitted except where they seem indi-

cated as an index of the degree of purity of any given fraction. With the exceptions to be noted, the lipoids from each of the three sources were extracted in similar manner. In Table I are given the chemical analyses of some of the fractions from beef brain No. I, the reactions to which are illustrated in the text and summarized in Table II.

I A. Drying and removal of the acetone soluble lipoids. One of the calf brains was dried by hot air and then extracted with acetone until the filtrate was colorless. The other three brains were treated with acetone directly without previous

TABLE I

Chemical Analyses of Lipoids from Beef Brain No. I (cf. Table II)*

EXP. NO.	DESCRIPTION OF LIPOID	CHEMICAL ANALYSIS							
		Mgm. Used	Cholesterol %		C %	P %	Nitrogen %		N:P
			Total	Free			Total	NH_2	
363	Ether soluble phosphatides (lecithins and cephalins)	254	0.31	0.03	60.9	3.5	1.99	0.98	1.2:1
360 382	Sphingomyelins	295	0.0	0.0	66.4	1.12	1.36	0.39	1.8:1
353 354 355 365	Mixed cerebrosides after four crystallizations	122	0.0	0.0	66.1	0.19	2.6	0.18	30:1
374	Kerasin	16.3	0.0	0.0	66.8	0.0	2.6	0.18	

* The chemical analyses were performed by Mary E. Gray, under the direction of Dr. Charles S. Robinson. The author welcomes this opportunity to thank both of these persons.

drying. The raw material from which the F.L.2 was obtained had been dried in vacuo and extracted with acetone.

I B. Extraction of the acetone insoluble lipoids from the dried and partially defatted residues. The residues from the calf brains were extracted with warm ether followed by hot pyridine. The residues from the beef brains were extracted with hot ethyl alcohol followed by warm ether. The residue from which the F.L.2 was obtained was extracted with benzol; the benzol extract was concentrated in vacuo and treated with acetone; the acetone insoluble material was dried in vacuo. This was the material donated by Dr. Klein for these studies.

I C. Removal of water soluble impurities from F.L.2. Before the F.L.2 was extracted further it was twice emulsified in salt water followed by precipitation with acetone, according to the method described by MacLean (25) for the removal

of water soluble impurities. The final precipitate was subjected to biologic assay. Since no difference was detected in the reactions of the tissues to F.L.2 before and after this procedure it was omitted in the extractions of the whole brains.

I D. Separation of the acetone insoluble lipoids into the ether insoluble, and ether soluble fractions.

D 1. The ether insoluble fraction. The alcoholic extracts of the two beef brains were concentrated by suction at room temperature and decanted. The residues were treated with warm ether until the supernatant fluid became colorless.

The ether extracts of the different brains were allowed to settle at 5°C. The residues were treated with ether until the supernatant fluid was colorless. The final residues from the calf brains were mixed with the pyridine extracts of their respective brains. The final residues from the beef brains, were added to the respective ether treated residues from the alcoholic extracts described in the preceding paragraph.

The dried lipoids from which the F.L.2 was prepared were dissolved in ether and treated like the ether extracts of the brains described above.

D 2. The ether soluble fraction. (This fraction was purified from F.L.2 and from beef brain No. I.) The filtrates from the alcoholic and ether extracts of the beef brain were carried separately through the stages of purification. After decantation of the ether insoluble fraction they were dried under suction at room temperature. The dried residues were repeatedly dissolved in ether, allowed to settle at 5°C., decanted and precipitated with acetone until no sediment occurred in the cold ether solutions. The final ether solution was used for the biologic assays of the ether soluble fraction (Tables I and II).

The mixed ether filtrates which were recovered in the separation of the ether insoluble fraction of F.L.2 were treated in the same manner as the ether solutions from beef brain No. I.

I E. Separation of the ether soluble fraction into the cephalins and the lecithins. (This was done only in the case of F.L.2.)

E 1. The cephalins. The purified ether soluble fraction of F.L.2 was dissolved in absolute alcohol over a water bath. The solution was cooled to room temperature and decanted. The residue was washed with alcohol, dissolved in ether and either: 1) re-precipitated with absolute alcohol at 5°C., or 2) precipitated with acetone followed by re-solution of the precipitate in heated alcohol. The process was repeated until the alcoholic filtrate was colorless. The final precipitate was used for the biologic assays of the purified cephalins.

E 2. The lecithins. The alcohol soluble filtrates left after extraction of the cephalins were mixed and precipitated with acetone. The precipitates were either: 1) dissolved in ether followed by an excess of absolute alcohol, or 2) dissolved in absolute alcohol directly. In either case the alcoholic solutions were repeatedly allowed to stand at 5°C., filtered, precipitated with acetone and re-dissolved in ether and alcohol or alcohol alone until no precipitate occurred in the cold. The final alcoholic solution was then packed in salted ice, centrifuged, decanted and

TABLE II

Tissue Reactions to Lipoids from Beef Brain No. I

EXP. NO.	DESCRIPTION OF FRACTION INJECTED	FIG. NOS. IN TEXT	DAYS SINCE INJECTION	PROPORTION OF CELLULAR TYPES IN THE INFILTRATIONS*						
				PMN	Phagocytic Mononuclear Cells					Fibroblasts
					Monocytes	Macrophages Characteristic of			Epithelioid	
						Inflammations	Phosphatides			
							Ether Soluble	Ether Insoluble		
	Ether Soluble Fraction									
348	Crude ether soluble phosphatides (lecithins and cephalins)	1, a, b	5	+	±			++++		
350	Above partially purified	2	5	±	±		++++	++	±	
363	Above further purified†	3 and 8, a, b, c	3	+++	+		++++			
	Ether Insoluble Fraction									
347	Crude ether insoluble fraction	4, a, b	4	++				++++		
351			3	±	±			++++		
375	Crude pyridine insoluble fraction (sphingomyelins)		3	+++				++++		
360	Purified sphingomyelins†	5, a, b, c, d, e	4	±				++++	±	+
382		9, a, b, c, d	3					++++		

370	Crude pyridine soluble fraction (cerebrosides)		4	++++	+++	++		+		
362	1st mother liquor from crystallization of cerebrosides	6	4	++++		++		+++		+
353	Cerebrosides (mixed) after four crystallizations†		3.5	++++		++			+	+++
354			5	++++		++			+	
355			5	+++		++				
365		11	3	++++	+	+				+
374	Phrenosin	13	3	+++	++	+++				++
374	Kerasin†	14, a, b	3	+++	+	+++			±	+++

* This tabulation is based upon estimates of the relative proportions of cellular types made from the supravital studies of the injected areas. Key: ± = very rare, + = few, ++ = moderate number, +++ = many, ++++ = great numbers and apparently the dominant cell present.

† The chemical analysis of this fraction is given in Table I.

precipitated with acetone. The precipitate was used for the biologic assays of the purified lecithins.

F 2. The galactolipids. (*This fraction was purified from all sources.*) The pyridine filtrates recovered from the sphingomyelins were precipitated in the cold with acetone and re-treated once in fresh pyridine. The second precipitates were crystallized from two to four times from a mixture of chloroform and ethyl alcohol. The final crystals were used for the biologic assays of purified mixed galactolipids (Tables I and II for the beef brains). The mother liquors and the different batches of crystals were subjected to biologic assay.

I G. Separation of the galactolipids into kerasin and phrenosin. (*This was done on F.L.2 and beef brain No. I.*) The purified crystals of mixed galactolipids were dissolved in 90 per cent acetone and allowed to stand at 37°C.

G 1. Phrenosin. The precipitate which occurred was repeatedly dissolved in a mixture of chloroform and glacial acetic acid, allowed to stand at 37°C., filtered and re-treated until the filtrate failed to cloud on cooling. The final precipitate was twice dissolved in a mixture of chloroform and acetone, kept at 37°C. and filtered. The precipitate from the second treatment was dissolved in 90 per cent acetone and allowed to crystallize slowly. The crystals were used for the biologic assay of purified phrenosin (Table II for beef brain No. I).

G 2. Kerasin. The filtrates recovered in the separation of phrenosin were allowed to stand at 5°C. The resultant precipitates were pooled and repeatedly dissolved in a mixture of chloroform and acetic acid, left at 37°C. over night, filtered, allowed to stand at 5°C., filtered, and the precipitates re-treated until sedimentation failed to occur at 37°C. Due to the small amount of material, the final precipitate obtained at 5° was not subjected to the further process of purification recommended by MacLean and MacLean (26), but was used at that stage for the biologic assay of purified kerasin (Tables I and II for beef brain No. I).

II. Biologic Assays. Throughout the course of the fractionations one hundred and twenty biologic assays were made with the different chemical groups of lipoids and their individual members, and also with the various incompletely fractionated groups and mother liquors. These assays consisted of supravital and fixed tissue studies of scrapings, films and blocks from subcutaneous areas previously injected with the material under examination. Guinea pigs were used for all of the assays. The findings, however, were repeated in many instances on mice and rabbits.

The substance to be injected was evaporated to dryness over a water bath in order to remove any lipoid solvents. It was then emulsified in either distilled water or physiologic saline in concentrations which varied from 0.1 to 3.0 per cent. The emulsions were sterilized with steam for two hours, and injected with sterile precautions.

Two to 4 cc. were given subcutaneously in the posterior surface of the thigh of a guinea pig. Care was taken not to produce too firm a bleb. A different emulsion was usually employed for each leg. A similar procedure was employed when rabbits were used. When repeated on mice, injections of 1–2 cc. were given in the subcutaneous tissues of the back and abdomen.

The effects of the injected material upon the phagocytic mononuclear cells were of particular interest. Therefore, the examinations of the experimental areas were made from 3 to 5 days after the injections, since the previous studies (35) had shown that to be the interval during which the most dominant effects upon those cells are to be expected following subcutaneous injections. The animals were sacrificed by administration of chloroform, ether, or illuminating gas, and the tissue studies followed immediately. A longitudinal incision was made across the area of injection. The skin was peeled back by traction, and, after gross examinations, scrapings were made of the under surface. These were touched to coverslips which were then inverted on slides previously coated with neutral red, or a mixture of neutral red and Janus green. The smears were rimmed with vaseline, placed in a hot box at about 37°C., and examined within half an hour. In the meantime the connective tissue overlying the muscle below was examined. A block of muscle with its overlying connective tissue was dropped in Bouin's fixative. Other bits of the filmy tissue were then gently spread on coverslips, moistened with serum from the cardiac cavity and inverted on slides stained with the supravital dyes.

The fixed tissues were carefully dehydrated and embedded in paraffin. The sections were cut at a thickness of three micra and stained lightly with H and E.

The supravitally stained smears and tissue films were examined for the number of cells present, the types of cells present, and the morphologic characteristics of each type, especially of the phagocytic mononuclear cells. The latter category includes observations of the size of the cells, the number, position and density of the nuclei, the presence of mitochondria, the size, shape, number, color and refractivity of the phagocytic vacuoles, and the presence of refractive unstained droplets. Since the latter could be stained with Sudan III, they are regarded as one of the acetone soluble fats and are simply called "fat" whenever discussed in the text.

EXPERIMENTAL DATA

(with pertinent literature)

The first studies in this series were made upon the product from the Wilson Laboratories, called F.L.2. This was the material which had given the characteristic phagocytic mononuclears described previously (Figs. 5, 6, 7 of reference 35) and which led to the present series of studies. Suspecting at the time that the size and phagocytic activity of the mononuclears were due entirely to the content of lecithin in the product, the first extractions were directed toward further purification of that phosphatide, and the by-products were discarded. As the lecithin became more highly purified, it became evident that the reaction to it was becoming more like that previously obtained with egg lecithin, and that the specific characteristics of the reactions to F.L.2

must have been due largely to the discarded impurities (Figs. 1, 2, 3). These were present in too small amounts to make extraction practicable, and therefore recourse was had directly to brains. The reactions to the ether soluble fractions of the F.L.2, however, were so specific, that it was considered unnecessary to repeat the studies with similar fractions from more than one brain.

Some of the extractions and corresponding biologic assays were therefore made of the F.L.2 only, and some of the beef or calf brains only, while others were repeated for each lot of material. The biologic reactions to any given chemical fraction proved to be identical irrespective of the source. The reactions are therefore classified according to their characteristics for each chemical fraction, and the members of that fraction, regardless of whether extractions and biologic studies of the particular fraction in question were carried out upon each experimental source. Information concerning the number of sources examined is, however, available in the outline of methods of extraction.

The reactions obtained with the various purified fractions from beef brain No. I are presented in Table II. They are characteristic of the reactions to the equivalent fractions from any of the sources. Table I gives the chemical analyses of some of the fractions listed in Table II.

I. Biologic reactions to the ether soluble fraction—the lecithins and cephalins. The subcutaneous reaction to the purified ether soluble fraction is characterized by the great number and phagocytic activity of the large mononuclear cells, by the rapid absorption and disappearance of both the injected material and the macrophages taking part in the absorption, and by the absence of fibrous residue. Supravitally the mononuclear cells vary in size from about 17 to 25 micra in diameter. They are very like the hyperactive macrophages commonly found in the reticulo-endothelial organs. They are never binucleated and the nucleus is usually centrally placed. They contain many phagocytic vacuoles which vary in size considerably and are of a red brown color (Figs. 2 and 3). They are more red than the otherwise similar vacuoles obtained after injections of egg lecithin. The vacuoles tend to be large on the third day after an injection, often attaining a diameter of as much as several micra, but they gradually become much smaller within the next few days, after which the cells rapidly disappear without any residual local reaction. The cells in the films often are

packed with unusually small even vacuoles diffusely scattered throughout the cytoplasm, but epithelioid or giant cells are never observed. The cells are richly supplied with mitochondria, about the size of those in blood monocytes. Rarely they contain a few unstained refractive droplets of fat which will stain with Sudan (Fig. 3). The rarity of this finding and its greater association with some extractions than with others suggest that it may be due to incomplete removal of other lipoids rather than characteristic of the ether soluble phosphatides themselves (see Table I and Fig. 3).

In sections of these areas, medium sized mononuclear cells are found in packed masses overlying the muscle and between the fibers, and are often lined between strands of fibrous tissue, but without increased fibrous reaction (Fig. 8, a, b). These cells have a slightly foamy cytoplasm and moderately vesicular nuclei. At times the cytoplasm is honeycombed but usually it is coarsely flocculent rather than definitely vacuolated (Fig. 8, c). It is slightly more basophilic than that of a blood monocyte. Mitotic figures occur frequently.

The mixed ether soluble lipoids obtained from the F.L.2 were further fractionated into the group containing the cephalins and that containing the lecithins. The biologic reactions to each of these groups are identical, and similar in every respect to the reaction to the mixed ether soluble lipoids described above in detail.

Detre and Sellei (9), Stasson and Billon (33), Kimmelstiel and Laas (20) and Sjovall (32) obtained very similar cellular reactions after injections of the preparations of lecithin which they used. As in the case of the lipoids studied here, Kimmelstiel and Laas found that the phagocytic mononuclears are smaller than after injections of the other lipoids which they employed, that some contain sudanophilic droplets and that their intake and output of lecithin is very rapid. Sjovall made a very comprehensive study, employing various routes of administration, dosages, intervals of time and histochemical methods, and came to the conclusion that the absorption of lecithin by the reticulo-endothelial system is so rapid that it is impossible to produce systemic accumulations of it by exogenous introduction alone. He believes that the intra-cellular deposits regularly pass through a sudanophilic phase before disappearance.

II. Biologic reactions to the ether insoluble fraction—the sphingomye-

lins and the galactolipids. This fraction produces reactions similar to those obtained with the F.L.2 alone before extraction of the ether insoluble contaminants (35). The reaction is very characteristic and sufficiently dominant that it colors the reaction to other lipoids when present in relatively small amounts (cf. Figs. 1, a, b and 4, a, b). This mixture was further fractionated into its two chemical groups, the sphingomyelins and the galactolipids, the former of which was found to be biologically active, and the latter, inactive.

Detailed description of the reaction to the ether insoluble fraction *in toto* is, therefore, omitted and given only for the reactions to the purified components of the mixture. It is interesting to note, however, that while the sphingomyelins compose the biologically active fraction with very distinctive characteristics, those characteristics are actually intensified in reactions to incompletely fractionated mixtures in which galactolipids are still present (Figs. 1, a, b; 4, a, b; 6; 7; 10, a, b). Experimental analysis of this observation has been presented elsewhere (37).

II A. Biologic reaction to the sphingomyelins. This fraction was found to be present only in traces in the F.L.2 and could therefore not be isolated from that source. The amount in calf brains also proved to be too small to make extraction practicable. Satisfactory amounts were, however, obtained from each of the beef brains and subjected to individual biologic assays. As in the case of the ether soluble phosphatides, this group of phosphatides calls forth great numbers of macrophages when injected subcutaneously, is quickly absorbed and disappears without fibrosis. Likewise, as in the case of the ether soluble phosphatides, the characteristic features of the reaction lie in the activity and absence of degenerative changes of the phagocytic mononuclear cells. Nevertheless, they differ markedly and strikingly from the macrophages called forth by the ether soluble phosphatides. Supravitally they are much larger, and contain much bigger phagocytic vacuoles (Fig. 5, a, b, c). The vacuoles are more uniform in size and shape, are slightly less red and pass through a colorless stage before disappearance, rather than simply becoming smaller in size as is the case of the cells which respond to the ether soluble phosphatides. The cells vary in size up to about 50 micra in diameter. The nuclei are usually eccentrically placed, slightly oval,

homogenous and but barely refractive. Binucleated cells are extremely rare. The phagocytic vacuoles are very remarkable. They are round, uniform in size and about 4 micra in diameter. For the first few days of the reaction they are uniformly deep brown-red or maroon (Fig. 5, a, b) but at the later stages of the reaction, when the cells have attained great proportions, they are often homogenously gray (Fig. 5, c). In either case they are only slightly refractive. At the later stages of the reaction the smears contain a variety of morphologic forms represented by cells which contain only red vacuoles, red vacuoles interspersed with gray ones, or only gray ones (Fig. 5, a, b, c, d, e. Also cf. Figs. 1, b; 4, b; and 6). From then on the cells rapidly disappear without any local residual reaction. The cytoplasm is a structureless gray and may contain many mitochondria about the size of those in a blood monocyte. The refractive droplets of sudanophilic material which are at times seen in the macrophages responding to the other group of phosphatides are never met in these cells. Epithelioid and giant cells are never met.

In sections of these areas, large mononuclear cells are found massed above and between the muscle fibers, and often so compressed against each other as to present a glandular appearance (Fig. 9, a, b). The nuclei are almost round and moderately vesicular with one or more nucleoli. Mitoses are infrequent and there is no evidence of the formation of fibrous tissue. The mononuclear cells are so large and so evenly honeycombed with large vacuoles that they look like the cells of the adrenal cortex, and the cytoplasm can be distinguished only with difficulty (Fig. 9, c, d). It is slightly basophilic.

By intravenous injections of sphingomyelin Beumer and Grüber (4) obtained scattered infiltrations of similar cells in various organs, and Ferraro (16) obtained generalized infiltrations comparable to those in Niemann Pick's disease. Tompkins (38) obtained results very similar to those of Ferraro by intravenous injections of the entire ether insoluble fraction of brain lipoids.

II B. Biologic reactions to the galactolipids. This fraction was present in appreciable amounts in the F.L.2 as well as in both the calf and beef brains, and extracts from each source were examined biologically. The mother liquors obtained in the course of crystallization were found to elicit the exaggerated reactions characteristic of

sphingomyelin and particularly of mixtures of galactolipids with phospholipids (Figs. 6; 10, a, b). Subcutaneous injections of purified crystals of the mixed galactolipids, however, invariably result only in inflammatory reactions with an active infiltration of polymorphonuclears and the formation of a capsule of fibrous tissue with little absorption of the encircled galactolipids. Any phagocytic mononuclear cells that do occur are typical of those about areas of inflammation (i.e., small, and filled with debris and fat) and in no way suggestive of the macrophages in areas injected with either group of the phosphatides. The fixed tissues corroborate the supravital findings (Figs. 11; 12, a, b).

In view of the fact that Gaucher's disease is characterized by the deposition of one of the galactolipids, kerasin [Lieb (23), Epstein (10) and Zehender (40)] and by the presence in the tissues of great numbers of foam cells [Bloom (5), Brile, Mandlebaum and Libman (7) and Horsley, Baker and Apperly (19)] it seemed indicated to fractionate the mixture of galactolipids into the components, phrenosin and kerasin, despite the fact that the mixture had proven inert. This was done in the case of the F.L.2 and beef brain No. I. The individual galactolipids from both sources are as inert as their mixture, and elicit a similar reaction (Figs. 13; 14, a, b).

Beumer and Fasold (3) failed to obtain histologic evidence of the presence of cerebrosides in the tissues after intravenous injections of the same, although they were able to demonstrate its presence chemically, and to determine that its absorption from the tissues is very slow. They found it necessary to add a protective protein for the injections. Kimmelstiel and Laas (20) were able to obtain foam cells from subcutaneous, intraperitoneal and intravenous injections of cerebrosides. They state, however, that the capacity of cerebrosides for cellular penetration is very slight. They found that the cells which responded to injections of experimental mixtures of the cerebrosides with other lipoids were similar to those obtained after injection of the cerebrosides alone, and they were able to inject the cerebrosides intravenously without a protective substance. In view of these findings, therefore, it seems that some chemical difference must exist between the preparation of cerebrosides employed by them and those used by Beumer and Fasold, and for the studies reported here.

DISCUSSION

These findings indicate that the biologic reaction to each chemical group of the brain lipoids studied is specific, and can be obtained free of characteristics due to other groups only when a given fraction has attained a high degree of purity. The early reactions are the same irrespective of the lipoid injected and are characterized by an influx of polymorphonuclear cells and monocytes. The specific characteristics of the reactions to each chemical group consist of the response of the phagocytic mononuclear cells. The phosphatides have the power to call forth these cells and are quickly absorbed without a residuum of fibrosis. The galactolipids on the other hand, have little effect upon these cells, are slowly absorbed and give rise to irritative reactions and the formation of a fibrous tissue capsule. In addition to the ability which the phosphatides have in common of stimulating an abundant infiltration of unusually phagocytic mononuclear cells, the characteristics of the cells and of their vacuoles are specific for the two chemical groups into which the phosphatides may be subdivided. The macrophages are smaller, contain smaller and different colored phagocytic vacuoles when stained supravitally, are much less foamy when fixed, and disappear more rapidly in areas injected with the ether soluble phosphatides, the glycerophosphatides, than with the ether insoluble ones, the sphingomyelins.

The explanation of these differences in reaction to the various chemical groups is not certain. That both groups of phosphatides stimulate the production of macrophages while the galactolipids are inert is probably due to some common characteristic of the phosphatides. That the macrophages which respond to the sphingomyelins differ morphologically from those which respond to the glycerophosphatides is probably inherent in specific chemical differences in the two groups of phosphatides which have been phagocytosed. The findings would seem to support the theory of Epstein (14) and of Kimmelstiel and Laas (20) that phosphatides have a high potency of penetration through cell membranes due to their physiochemical characteristics, while other lipoids have a low capacity of penetration and achieve entrance only by virtue of admixture with phospholipids. It is, however, not evident if these differences in permeability explain the difference in the ability of the two lipoidal groups to stimulate infiltrations of cells which can phagocytose them. Once within the

cells, however, the galactolipids produce changes which simulate those due to the sphingomyelins alone (37) while the glycerophosphatides, on the other hand, produce a different morphologic picture.

It seems likely, therefore, from these various considerations, that the power of penetration on the part of the brain phosphatides is due to the one group that they all have in common, the phosphoric acid radical, or the (OH) radical, according to Epstein (14), while the specific characteristics of the phagocytic vacuoles, once the substances have penetrated, are due to other chemical groups forming the specific lipoids. The fatty acids of brain lecithins and cephalins have been shown to be closely related, Thudichum (34), MacLean and MacLean (26). The morphology of the cells responding to those substances is similar. The sphingomyelins and galactolipids have a fatty acid in common, the sphingosine radical, and the relationship becomes even closer in the case of kerasin, sphingosine-lingoceric. The morphology of the macrophages responding to kerasin, once it has entered the cells, is very similar to that due to the sphingomyelins alone (37).

That the cells elicited by the highly purified ether soluble phosphatides from brains should be practically identical to those elicited by egg lecithin (35) lends added support to the concept that the glycerophosphatides normally present in animal sources stimulate the production and phagocytic activity of macrophages without resultant degenerative effects upon them (35).

The production of epithelioid cells by Sabin and her co-workers (29) (30) (31), however, by injections of the glycerophosphatides from acid fast bacilli obviously indicates that the stimulative effects exerted by the phosphatides of brains and of eggs are not necessarily characteristic of other phosphatide linkages. The dextrorotatory phthioic acid in the phosphatide from tubercle bacilli was found to be the component responsible for the formation of epithelioid cells by that phosphatide (31) (2). Yet stearic acid, which Gray (17) has found will also produce epithelioid cells when injected as the synthetic triglyceride, loses that effect in linkage with phosphoric acid as found in brains and eggs, while phthioic acid continues to exert its effect even in that linkage.

Since the chemical studies of Epstein and Lorenz (12), Tropp and

Eckardt, (39), Klenk (21), Chargaff (8) and others agree in according sphingomyelin the dominant part in the abnormal storage of lipoids in Niemann Pick's disease, even while they differ as to its singleness, it is to be expected that the foam cells in that disease should bear resemblance to those obtained by experimental injections of sphingomyelin. The descriptions by Bloom (6) and Apt and Bloom (1) of the cells in fresh preparations from a case of Niemann Pick's disease are very similar to cells stained supravitally from the subcutaneous areas injected with the sphingomyelins, and their descriptions of the fixed cells, as well as those of Pick (27), Epstein (13) and others are entirely comparable to the fixed foam cells in the experimental subcutaneous tissues. It seems probable that such minor differences as do occur in the cellular morphology from case to case are dependent upon the variable proportions of accompanying lipoids which have been demonstrated by the chemical analyses cited above.

While they differ in certain very definite respects, descriptions of the cells characteristic of Gaucher's disease [Erf (15), Horseley, Baker and Apperby (19), Bloom (5), Marchand (24), Riesel (28), Pick (27), Hoffman and Maker (18) and Zehender (40)] are similar in many ways to descriptions of the cells characteristic of Niemann Pick's disease. They are like the cells obtained experimentally with the ether insoluble fractions of the brain lipoids. Since Epstein and Lorenz (11) have found an increase in phospholipids as well as of kerasin in the tissues in most cases of Gaucher's disease, and Tompkins (37) has shown that the reaction to mixtures of galactolipids and phosphatides from brains have striking similarities to the reactions to sphingomyelins alone, it is probable that the cells characteristic of Gaucher's disease are dependent in large measure upon admixture of the kerasin with phospholipids.

SUMMARY

1. The reaction of the subcutaneous tissues to injections of the acetone insoluble lipoids of beef brains has been studied in rabbits, guinea pigs and mice.

2. The reaction to the various chemical fractions of these lipoids is the same for each species.

3. The reactions are specific for each chemical fraction of these lipoids.

4. The phospholipids call forth infiltrations of phagocytic mononuclear cells and are quickly absorbed without residual reaction.

5. The phagocytic mononuclears which respond to ether soluble phosphatides (the glycerophosphatides) differ morphologically from those which respond to the ether insoluble phosphatides (the sphingomyelins).

6. The galactolipids stimulate merely irritative reactions characterized by infiltrations of granulocytes, poor absorption and encapsulation with connective tissue.

7. The findings are discussed from the standpoint of their correlations with a) the chemical relationship between the lipoids and b) the histo-pathology of Gaucher's, and Niemann Pick's disease.

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PLATES

PLATE I

Figures 1–7 inclusive are from colored drawings of cells stained supravitally with neutral red. All are drawn to the same scale. Magnification × 1121. Vacuoles which did not stain appear pearl gray in the reproductions just as they appeared through the microscope. Those which took the dye appear more or less dark and dense in the reproductions, dependent upon the intensity with which they were stained.

FIGURE 1, a, b. Exp. 348. Macrophages from an area injected 5 days previously with the crude ether soluble fraction from beef brain No. 1. The similarities to Figs. 4 and 6 show the influence exerted by the ether insoluble impurities which were present, i.e., large size of cells and vacuoles, and the occurrence of gray as well as of stained vacuoles. The color of the stained vacuoles was a darker and clearer red than that of cells due to the ether insoluble lipids.

FIGURE 2. Exp. 350. Macrophage from an area injected 5 days previously with a more highly purified fraction from the same material as was used for Fig. 1. With removal of most of the ether insoluble impurities, the morphology characteristic of response to the ether soluble phosphatides became distinct, i.e., smaller dimensions of cell and vacuoles, deeper red of vacuoles, and absence of unstained gray ones.

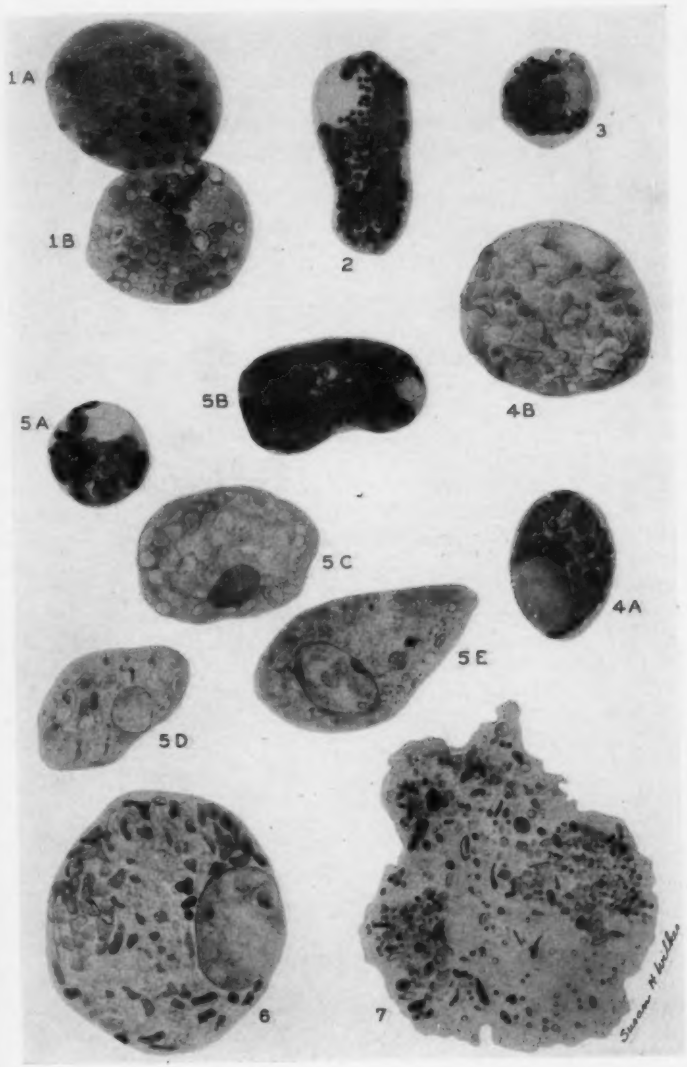
FIGURE 3. Exp. 363. Macrophage from an area injected 3 days previously with a still more highly purified fraction (Table I) of the same material as was used for Fig. 2. Cholesterol was still present and probably accounts for the highly refractive, unstained droplets in addition to those which took the supravital dye.

FIGURE 4, a, b. Exp. 351. Macrophages from an area injected 3 days previously with the crude ether insoluble fraction from beef brain No. 1. The cells were like those due to the sphingomyelins alone (Fig. 5, a, b, c) except that they ultimately attained greater dimensions. They were also similar to the cell shown in Fig. 6, and, except for lighter vacuoles with a somewhat more brownish tint, to those shown in Fig. 1, each of which was dominantly influenced by the ether insoluble lipoids present in the material injected.

FIGURE 5, a, b, c, d, e. Exp. 360. Macrophages from an area injected 4 days previously with sphingomyelins (Table I) extracted from the ether insoluble fraction which elicited the cells shown in Fig. 4. Note the uniformity of size and color of the phagocytic vacuoles in any given cell, their capacity to stain in the smaller cells (a, b), their failure to stain in the larger ones (c, d) and their ultimate disappearance (e).

FIGURE 6. Exp. 362. Macrophage from an area injected 4 days previously with the first mother liquor obtained in crystallization of the cerebrosides from the ether insoluble fraction which elicited the cells shown in Fig. 4. This cell was stimulated by the presence of sphingomyelins in the mother liquor, but was larger than those due to sphingomyelins alone (Fig. 5, a, b, c). It is similar to the cells shown in Figs. 1 and 4, which were also under the influence of cerebrosides in addition to phosphatides.

FIGURE 7. Macrophage produced by the subcutaneous injection of a mouse (AF No. 49) 8 days previously with an experimental mixture of kerasin and the ether soluble phosphatides used for Fig. 3 (0.01 gm.: 0.02 gm.). Note the size of the cell and its similarities to those shown in Figs. 1, 4 and 6, which were elicited by mixtures in which sphingomyelins were still present. The color of the stained vacuoles was the same as that due to the ether soluble phosphatides alone (cf. Figs. 2 and 3). There were also many gray vacuoles, and a few highly refractive unstained ones. The rods in the cell are believed to be crystals of the kerasin.



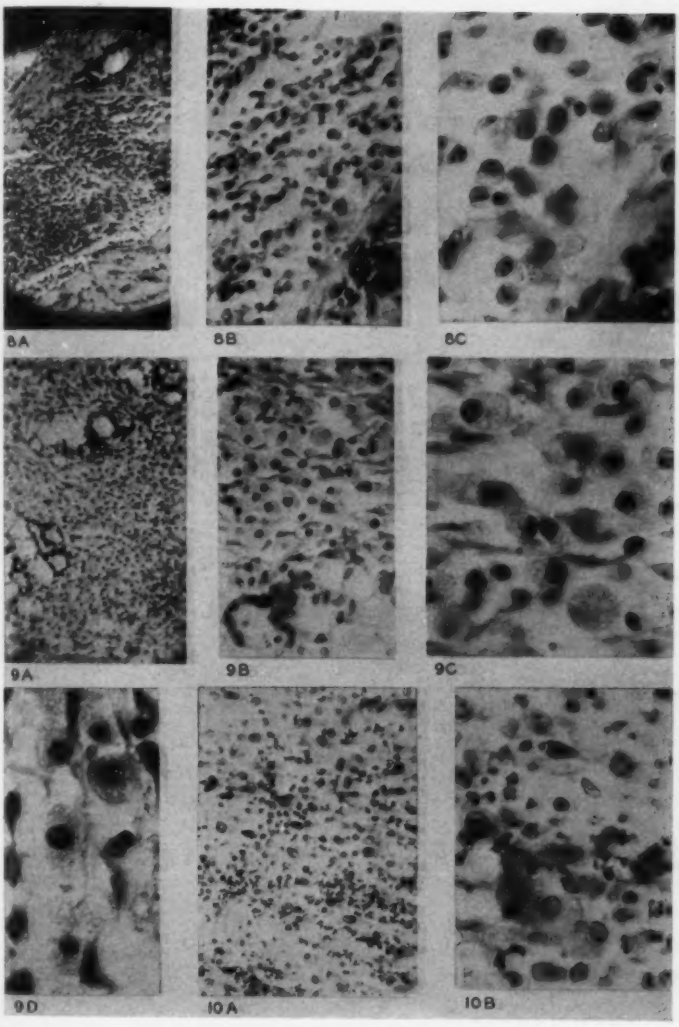
FIGS. 1-7

PLATE II

FIGURE 8, a, b, c. Exp. 363. Fixed tissue from the same area as Fig. 3. Illustrated in order to show the abundance (a) and uniformity (b) of the reaction of the macrophages to the ether soluble phosphatides, and their characteristics after fixation (c). Magnifications × 82, 228 and 410, respectively.

FIGURE 9, a, b, c, d. Exp. 382. Fixed tissue from an area injected 3 days previously with the same lot of sphingomyelins that elicited the cells shown in Fig. 5, a, b, c, d, e. Illustrated in order to show the abundance (a) and uniformity (b) of the reactions of the macrophages to these phosphatides and their characteristics after fixation (c, d). Note that while the abundance of the reaction is the same to both groups of phosphatides, the cells responding to the sphingomyelins are the larger and are vacuolated (cf. Fig. 8, b, c with Fig. 9, b, c). Magnifications × 82, 228 and 410, respectively.

FIGURE 10, a, b. Exp. 378. Fixed tissue from an area injected 3 days previously with the first mother liquor from the crystallization of the cerebrosides of beef brain No. 2. The reaction was due to a mixture of the cerebrosides with sphingomyelins, and while dominately irritative in nature, was characterized also by the presence of large vacuolated macrophages. Magnifications × 228 and 410, respectively.



FIGS. 8–10

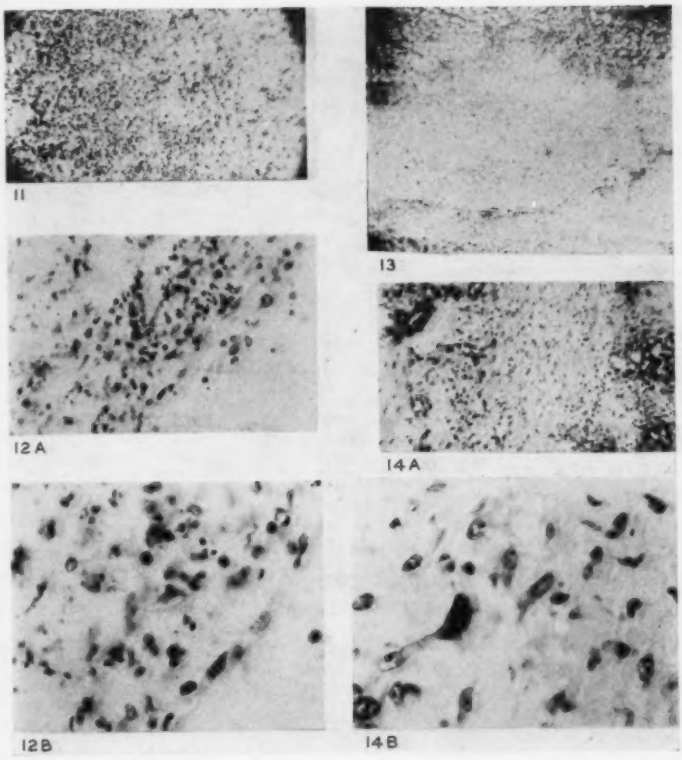
PLATE III

FIGURE 11. Exp. 365. Reaction to the purified mixed cerebrosides from beef brain No. I (Table I) injected 3 days previously. Compare to Figs. 8, a; 9, a; 10, a. Note the absence of macrophages and the purely irritative reaction accompanied by fibrosis. Magnification × 82.

FIGURE 12, a, b. Exp. 372. Reaction to the purified mixed cerebrosides from beef brain No. 2 injected 3 days previously. Compare to Figs. 8, a; 9, a; 10, a. Note the absence of macrophages and the purely irritative reaction accompanied by fibrosis. Magnification × 228 and 410, respectively.

FIGURE 13. Exp. 334. Reaction 52 hours after injection of phrenosin from F.L.2. A fibrous capsule was forming about the structureless mass of phrenosin and absorption was negligible. Magnification × 24.

FIGURE 14, a, b. Exp. 374. Reaction 3 days after injection of kerasin from beef brain No. I (Table I). The structureless masses of kerasin were being slowly invaded by fibrous tissue with a rare macrophage. Compare with the reactions to phosphatides, Figs. 8, c and 9, c. Magnifications × 82 and 410, respectively.



FIGS. 11–14

DEMONSTRATION OF THE BASILAR ARTERY AND ITS BRANCHES WITH THOROTRAST

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Since the introduction of cerebral angiography by Moniz, arteries and veins in many parts of the body have been visualized by radiopaque substances. Moniz used 25 per cent sodium iodide, but many substances have also been used, and all have undesirable features. Several years ago, thorotrast, a colloidal suspension of thorium dioxide, was introduced as an agent suitable for angiography. The main objection to the use of this chemical is its mild radioactivity. The material is poorly excreted, and is largely stored in the bones and reticulo-endothelial systems but it is now generally agreed that amounts up to 25 c.c. are probably harmless.

One excellent feature of this preparation is its apparent freedom from causing immediate reactions. Frequently two injections into the internal carotid artery of 10 c.c. each, and even 3 injections of 8 c.c. each have been given with no reaction except that in an occasional case a rectal temperature of 101°F for eight hours followed. There have been no instances of headaches, convulsions, thrombus formation, emboli, hemorrhage, or other complications following injections up to 25 c.c.

As far as I have been able to determine, there have been no previous reports of visualization of the basilar artery during life. Consequently, there was no information as to how the cells of the brain stem would react to thorotrast. However, the majority of authors on this subject have not recorded any damage to the substance of the cerebral cortex following carotid artery injections by thorotrast, and I have seen none, following demonstration of the carotid system. Therefore it was felt that there was little reason to suspect that the brain stem would react any differently than do other parts of the brain. With

this in mind, it was decided that demonstratoin of the basilar artery might be feasible, and that should a patient be encountered in whom a pathological process of this vessel entered into the differential diagnosis, visualization might be useful. Temporary occlusion of one vertebral artery is of course necessary for about a minute pending the taking of x-rays.

Case A. W. Colored Male, 40 years of age. Baltimore City Hospitals 64809. Admitted April 2, 1941.

The patient first came under observation in 1932 when he went to the Out-Patient Department of the Johns Hopkins Hospital complaining of a sore throat. At that time a diagnosis of gummata of the tonsil and larynx, central nervous system syphilis and right hemiparesis was made. Under appropriate treatment the gummata healed, and he was treated at irregular intervals for several years. In 1938, he had another cerebrovascular accident, resulting in a transitory right hemiplegia and, in 1939, a left hemiplegia. Both of these cleared up promptly and completely.

The patient was admitted to the Johns Hopkins Hospital (unit no. 224600) on February 16, 1941. Four days before admission, he had had a sudden severe pain in the back of his head, followed by a generalized headache and stiffness of the neck. Almost immediately he noticed he was unable to swallow, and that he could not speak above a whisper. Until his entrance into the hospital, his condition had not changed.

On admission, his temperature was 102°F. The neck was stiff and hyperextended. Kernig's sign was positive bilaterally. Speech was dysarthric and swallowing almost impossible. The examination was otherwise negative except for a congenitally deformed left leg.

A lumbar puncture disclosed grossly bloody spinal fluid, containing 37,400 red cells per cu. mm. The supernatant fluid was xanthochromic. The serologic tests for syphilis in the blood and spinal fluid were negative.

The patient remained in the hospital for fifteen days. The temperature gradually fell to normal, and in a week, the cervical rigidity disappeared. There was no improvement in speech or swallowing. He was discharged April 1st, 1941.

The patient was admitted to the Baltimore City Hospitals on the following day. Additional findings described at this time were the presence of a jaw jerk, absence of the gag reflex, weakness of the sternocleidomastoid and trapezius muscles and inability to protrude the tongue fully. The nose and throat consultant found the vocal cords paralyzed. The remainder of the neurological examination was negative. Because of the spastic jaw, laryngeal and tongue muscles without signs of atrophy, the diagnosis of pseudobulbar palsy was made.

The usual laboratory studies were negative except for a positive serologic test for syphilis. A lumbar puncture revealed that the pressure of the spinal fluid was 150 mm. of water. The fluid was clear and contained no cells. The Pandy and

Wassermann reactions were negative. X-rays of the skull showed no abnormalities.

While in the hospital he had a few attacks of bifrontal headaches. These were not severe and were easily relieved. Once he complained of pain in the left temporal region which lasted about a day.

The question of an aneurysm of the basilar artery was raised because of the spontaneous subarachnoid hemorrhage. There were no localizing signs, except the hoarseness, difficulty in swallowing and poor voluntary movement of the tongue. It seemed hardly conceivable that a person could survive a primary hemorrhage in the substance of the brain stem which ruptured into the subarachnoid space, as such lesions are nearly always fatal. On the other hand, the patient might have a leaking aneurysm, producing subarachnoid hemorrhage, and at the same time causing local damage to the medullary tracts and resulting in a pseudobulbar palsy. Because this could not be excluded, it was decided to attempt a demonstration of the basilar artery.

METHOD OF VISUALIZATION OF BASILAR ARTERY

It was decided to expose the vertebral artery by the anterior approach where the artery enters the vertebral foramen. Under avertin and local anesthesia an incision was started on the right about 1 cm. above the right clavicle and extended superiorly for 6 cm. parallel to the inner margin of the sternocleidomastoid muscle. The platysma and superficial fascia were divided. The sternocleidomastoid muscle was freed and retracted laterally. The structures in the carotid sheath were retracted medially. The dissection was continued until the anterior scalenus muscle was found. This was kept laterally and as the body of the sixth cervical vertebra came into view, the right vertebral artery was seen. This was freed from the surrounding tissue and followed until its origin from the subclavian artery was demonstrated. A broad catgut ligature was then passed under the artery. The ends were left long and not tied. The function of the ligature was to occlude the artery temporarily when the thorotrast was being injected (Fig. 1).

The patient (remaining draped) was then rolled on the operating table to the X-ray department. An 18-gauge lumbar puncture needle to cannulate the artery had been previously bent at right angles with the bevel up. A straight needle would not have been suitable for the purpose because the vessel lies in the depth of the wound and it cannot be pulled out of the incision. It is necessary to get the needle well up

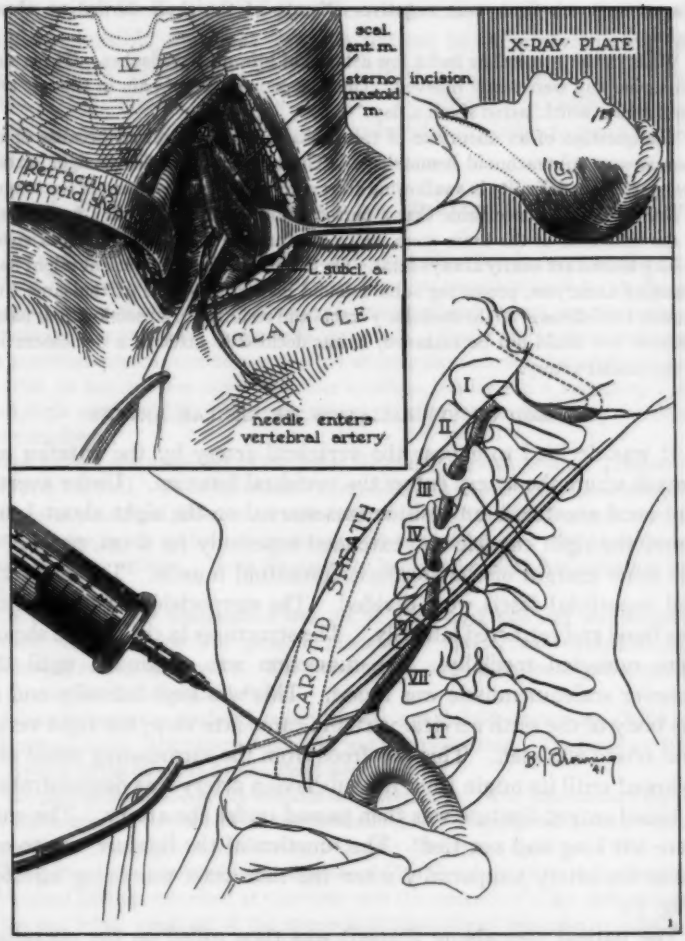

FIG. 1. Sketches showing the operative approach, the structures encountered in the neck, the position of the needle in the vertebral artery and the location of the x-ray cassette when a lateral view of the head is taken, to show the position of the basilar artery.

into the vessel yet avoid puncturing the opposite wall. This can be accomplished only if the needle is placed along the same axis as the vessel. The bent needle allows the lower half to parallel the course of the vessel while the upper half projects through the incision, and a syringe, containing the thorotrast, can easily be attached.

When the x-ray apparatus had been adjusted, the tube warmed and ready for instant use, the bent needle was inserted into the vertebral artery. A syringe containing 10 c.c. of thorotrast had been attached to the needle, and as soon as a pulsating stream of blood returned into the syringe the vertebral artery was occluded inferiorly by pulling together the ends of the previously placed ligature. Then the thorotrast was injected as rapidly as possible. As the last drops were passing out of the syringe, the technician was signalled and the picture was secured. Keeping the ligature tight, the assistant at the head switched cassettes and a second plate was exposed. This change took about 3 seconds.

The ligature was then released and a fresh syringe with 10 c.c. of thorotrast was attached to the needle. The latter had not been withdrawn from the vessel during this change. Meanwhile, the x-ray tube was shifted in order to take an anterior-posterior film. A third plate holder was slipped under the patient's head at the same time. The entire procedure took perhaps a minute. The artery was again occluded, the thorotrast injected and a film was taken as the last of the material left the syringe.

Details of the x-ray technique were as follows: A Coolidge XB 15 tube with a wide cone was used. It was placed 25 inches from the patient's head. Fifty kilovolts were delivered against the target. The exposure was $\frac{1}{2}$ second. A Bucky diaphragm was not used.

The needle was then withdrawn from the artery, and there was no bleeding. The wound was dry so the superficial fascia was approximated by a line of catgut sutures, and a second line of subcuticular stitches closed the incision. During the entire procedure, there was no change in the patient's pulse, respiration or general condition. His post operative course was entirely uneventful. There was no rise in temperature, the wound healed *per primum* and he was up, eating a ward diet on the third day after operation. Until the present he had

been entirely well. Since no operable lesion was found, no additional therapy was undertaken.

DISCUSSION

The x-ray film of the lateral aspect of the skull showed the right vertebral and basilar arteries clearly. Many small vessels over the

FIG. 2. Lateral view of the skull showing the position of the vertebral, basilar, cerebellar and posterior cerebral arteries. The bulbous ending of the basilar artery may be a small aneurysm. Note the absence of the posterior communicating arteries.

cerebellum were visualized, and about 4 cm. of the proximal portion of the posterior cerebral arteries were seen clearly. Instead of the basilar artery ending in a bifurcation which is the usual origin for the posterior arteries, there was a small bulbous projection about 0.5 cm. beyond these vessels. It was suggested that this might be a small aneurysm, since they are common in this location. However, since there are no other living controls, this is only a speculation (Fig. 2).

The second lateral plate showed thorotrast in the ends of the cerebellar and posterior cerebral arteries, and some in the dural sinuses. The anterior-posterior plate showed an end-on view of these arteries, with fanning out to both sides of the cerebellum (Fig. 3).

FIG. 3. Anterior-posterior view taken of the second injection of thorotrast. The photographs were retouched for reproduction to emphasize the main structures.

It should be noted that the posterior communicating arteries were not demonstrated. This has been noticed also when the internal carotid arteries have been injected. It would seem that wherever a stream of blood is flowing into the circle of Willis, that vessel has

never been visualized. Blood comes into the circle from either end of the communicating arteries, so that the higher pressure in the carotid artery forces blood into the communicating vessels which prevents the thorotrast from filling them. Neither anterior nor posterior communicating arteries have been demonstrated in any of my injections of the internal carotid or basilar arteries.

Several points might be mentioned which must be observed to obtain clear films. First the vessel must be completely occluded below the site of injection. If this is not done, the radiopaque material is diluted and diffused so rapidly that little or no detail is obtained. This holds true for carotid as well as vertebral injections. A second point is, that the needle must be large and the material injected as rapidly as possible. An 18-gauge needle can be pushed through arterial walls and withdrawn without any bleeding. The needle should be inserted at about a 30-degree angle to prevent puncture of the opposite wall, and to allow the arterial wall to contract more advantageously on withdrawal. Only once has bleeding followed removal of the needle and this was in a 70-year-old woman in whom the wall of the carotid artery was extremely sclerotic. Pressure over the puncture site for a few minutes controlled the hemorrhage.

A third point to be observed is that a large x-ray tube should be used, since the picture must be taken rapidly. A portable machine is not suitable; patients should always be taken to the x-ray department where heavy duty tubes are available. It might be supposed that wound contamination might occur but this procedure has been used in many cases of carotid artery injections without any infections.

Finally, it is necessary to have several people cooperating as a team. The technician taking the films must be ready to snap them at the signal from the operator. At the instant of completion, an assistant at the head must change the cassettes, since the thorotrast moves on rapidly, and no detail is secured after a few seconds.

As a maximum of 25 c.c. of thorotrast can be used per patient, good films must be obtained the first time if more than one vessel is to be injected. After some early failures, it is now possible to secure good films fairly constantly.

SUMMARY

The basilar artery and its branches were successfully demonstrated in a living human subject by means of thorotrast. There were no untoward reactions. The procedure should be considered in patients where the differential diagnosis includes aneurysms or defects of these vessels.

The author wishes to express his appreciation to Dr. William Cheney of the Surgical Staff and Mr. Charles Linder of the X-ray Department of the Baltimore City Hospitals for their help in securing the films.

ANTERIOR COMMISSURE TENDON OF THE LARYNX

Its Significance in the Laryngofissure Operation. Preliminary Note

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Malignant growths of the larynx are usually squamous cell carcinomata arising from the squamous epithelium of the anterior third of the vocal cords. These tumors grow slowly, progress chiefly by extension, and metastasize late. Hoarseness is usually the first symptom.

When a laryngeal growth is to be removed surgically, two types of operation are available: (1) The entire larynx may be removed (laryngectomy), or (2) The thyroid cartilage may be split open and the growth removed together with the affected vocal cord and, in certain instances, part of the thyroid cartilage (laryngofissure).

The purpose of this note is to direct attention to an anatomical structure which may have an important bearing upon the success of the laryngofissure operation. As far as I can discover, this structure has not previously been described.

The vocal cords at their anterior commissure are attached to the thyroid cartilage by a tendon which binds the anterior commissure firmly to the cartilage (Figure 1). This tendon, beginning above the false cords and continuing down below the attachment of the true cords, extends almost the entire length of the thyroid cartilage. Laterally, the perichondrium of the thyroid cartilage is not firmly attached and may be stripped back easily, but there is no line of cleavage where the anterior commissure tendon joins the cartilage.

The presence of the anterior tendon and its extension into the thyroid cartilage may explain many of the recurrences, or rather continuations of carcinomata of the vocal cords following the laryngofissure operation. In this operation the thyroid cartilage is split in the mid-line

and the anterior tendon is cut through but not removed. Tumor cells which are extending along the tendon may therefore be left behind.

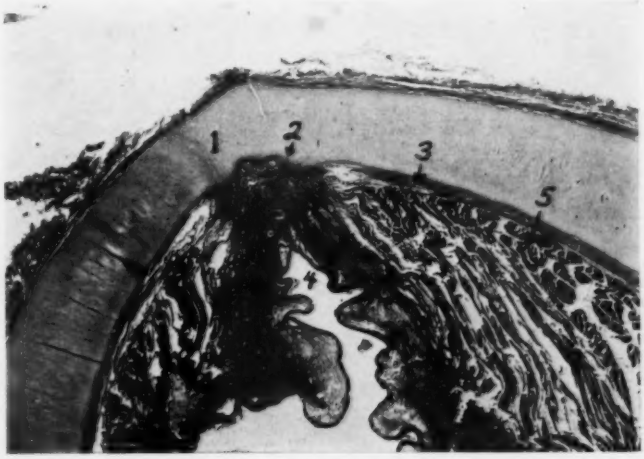

FIG. 1. VIEW OF ANTERIOR COMMISSURE

Magnification × 10. *1*, thyroid cartilage; *2*, tendinous attachment of vocal cords to cartilage; *3*, perichondrium of thyroid cartilage; *4*, anterior meeting of epithelium of vocal cords; *5*, muscle fibres of vocal cords.

On the basis of this anatomical study it would appear that in the laryngofissure operation it is always desirable to remove along with the diseased vocal cord the anterior ridge or crista of the thyroid cartilage and the anterior portion of the healthy cord.

PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, November 10, 1941

The President of the Society, Dr. Frank B. Walsh, presided. Abstracts of the papers and discussions follow:

Successful Complete Construction of an Extra-thoracic Esophagus: Case Presentation.

John Staige Davis and Edward S. Stafford (Department of Surgery.)

L. D., a white girl aged 2 years and 11 months, entered the Johns Hopkins Hospital October 8, 1928, three weeks after swallowing concentrated lye. Complete cicatricial stenosis of the esophagus had occurred. Gastrostomy was performed on the following day. During the next eighteen months numerous attacks of aspiration pneumonia proved nearly fatal, but the establishment of a cervical esophageal fistula in June 1930 was followed by improvement in general health and resumption of normal growth.

The construction of a new esophagus was begun in February 1940, and the task is now accomplished. A tube of full thickness skin was made from the anterior chest wall, and the resultant defect in the covering of the chest was roofed over by the transfer of pedicled tube-flap grafts from the flanks. The skin-tube was joined directly to the cervical fistula. The final step in the procedure was the connection of the lower end of the skin tube to the stomach, and for this purpose a segment of jejunum was isolated, brought through the transverse mesocolon and the gastro-colic ligament, and interpolated between skin-tube and stomach.

Fluid and solid food taken by mouth now pass quickly and directly to the stomach. The gastrostomy has not been allowed to close yet, since lack of experience in the use of an extra-thoracic esophagus makes the gastrostomy a desirable safety factor. Healing of the new esophagus is complete except for a tiny fistula situated at the point of anastomosis between skin-tube and jejunum. This is slowly closing.

Among the many interesting facts about this patient are the duration of her hospitalization (2036 days), the cost of her hospital care, which was borne entirely by the Johns Hopkins Hospital ($9912.00), the number of occasions on which general anesthesia was administered (67 times), and the complete feeding of this patient per gastrostomy tube for over 13 years.

A detailed report of the surgical procedures will be made later.

Discussion

Dr. Edwin N. Broyles said that it was unusual to find a complete closure of the esophagus resulting from stricture caused by swallowing lye. In this case, how-

ever, repeated efforts to pass a thread through the esophagus had been unsuccessful. The construction of the pharyngeal fistula by Dr. Davis was a most important life-saving measure.

Dr. Edwards A. Park asked what composed the lining of the new skin tube.

Dr. Davis said that the tube was lined with whole skin.

Nutrition in Public Health Practice as Illustrated by some Recent Studies in France. JOHN B. YOUMANS. (Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.)

Responsibility for developing and directing work in nutrition of the public, in which many agencies and organizations are interested, belongs to the public health departments through their division of nutrition. The latter operate in various phases of nutrition often in a cooperative or advisory way but guided by a function particularly their own, namely an epidemiological service. This epidemiological service involves surveys of nutrition. These may be of many kinds but the one most used at present includes (1) a study of the dietary of the subjects, and (2) individual examinations including laboratory tests, of which a number are available for detecting deficiencies of various nutritive factors in the earliest stages.

Such a survey was employed as one means of studying the state of nutrition in France in the winter and spring of 1941. The diet study disclosed shortages of vitamins A and C and riboflavin. Physical and laboratory examinations supported the dietary observations and although frank, fully developed cases of deficiency were uncommon there was a rather high incidence of mild grades of vitamin A and C deficiency, riboflavin deficiency and anemia. A considerable amount of rickets was encountered but this was believed not to be due to the current food shortage.

The foregoing findings apply to the period of the study only and subsequent developments will depend upon circumstances. Later surveys are expected to chart the course of the nutrition of the population.

DISCUSSION

Dr. G. Canby Robinson said that it was interesting to hear a scientific evaluation of the state of nutrition in France. He had just received a letter from former President Hoover saying that Americans would have to help out in the present situation or regret it later. He said it would be of value to future investigators to have this study which had been made at the beginning of the food shortage. Dr. Youmans' observations appear to indicate that there is a large margin of safety in nutrition to account for the delay in the development of deficiency diseases.

Dr. Edwards A. Park spoke of the great difficulty of making an accurate clinical diagnosis of deficiency states. He said that there were many cases of scurvy, rickets, and vitamin A deficiency in children which could be detected only by post mortem studies. He believed that perhaps the best way to detect minor degrees of deficiency was by adopting the old formula "Tell me what you eat, and I'll tell you what you are."

Dr. Maxwell M. Wintrobe said that the margin of safety mentioned by Dr. Robinson represented a large and essentially unexplored field between the taking of a poor diet and the development of the clinical syndromes which we have learned to associate with various nutritional deficiencies. As yet we know very little about the true margin of safety.

Dr. L. Emmett Holt asked Dr. Youmans whether he would care to contrast his findings in France with those of the similar study which he had made in the Nashville area.

Dr. Youmans remarked that it was very difficult to convey to an audience a correct idea as to the state of nutrition in France. He said that before the war, France was perhaps the best fed nation in the world, and that the French people were unusually thrifty. He felt that it was very likely, therefore, that the effects of food deficiencies upon them might be less, or might be slower in developing, than would be the case in a less well-fed nation. He pointed out the differences due to age; children show some deficiencies not observable in adults. He said that deficiencies may result from an upsetting of the normal balance of diet as well as from the actual lack of necessary foods. For these reasons, he felt that no general statement about a margin of safety could be made. Dr. Youmans observed that the French children did show marked retardation according to our standards, but probably less than would be shown by American children with similar deprivations. He said that nutrition studies will always require history-taking as well as objective investigations.

In reply to Dr. Holt's question, Dr. Youmans said that in the Nashville area he had found vitamin A and C deficiencies matching those found in France, and an even higher incidence of protein deficiency and pellagra.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Clinical Aspects of the Electrocardiogram. By HAROLD E. B. PARDEE. Fourth Edition. 434 pp. Illus. $5.75. *Paul B. Hoeber, New York and London, 1941.*

Since the publication of the last edition of this book in 1933 the use of precordial leads has added greatly to the diagnostic value of the electrocardiogram. The author clearly sets forth the differences between and significance of the various precordial leads. Condensed tables of the normal measurements of the waves in the most important precordial leads are presented. Other new material includes descriptions of parasystole and electrical alternans, and new information concerning the effect of a variety of diseases and metabolic disturbances upon the electrocardiogram. The book provides full and leisurely discussion of many difficult points which arise in electrocardiographic interpretation. It is perhaps better suited for use by the trained electrocardiographer than by the beginner.

C. B. T.

Fractures and Dislocations. By KENNETH M. LEWIS. 217 pp. $2.00. *Oxford University Press, London, New York, Toronto, 1941.* (*Oxford Medical Outline Series.*)

This new book is essentially a condensation of the standard text books on fractures. The subject matter is presented in more or less tabulated form and without illustrations. In addition to acute fractures and dislocations, related subjects, such as recurrent dislocation of the shoulder, spondylolisthesis, Kümmel's disease, and Osgood-Schlotter's disease, are discussed briefly.

Chapter three deals with delayed union and non-union, as if they were the same clinical entity rather than two distinctly different complications with distinctly different indications for treatment.

The author recommends the early resumption of upright posture in compression fractures of the dorsolumbar spine (after one to two weeks), and the use of the so-called hanging cast treatment for fractures of the shaft of the humerus. Since both of these measures have been found to yield a high percentage of unnecessary complications, it would perhaps have been better to urge more conservative treatment.

The presentation of the subject matter follows very closely the arrangement used in the larger text books on fractures. General considerations and principles of treatment of fractures in general are dealt with first, then comes the consideration of each specific fracture in topographical order.

Interspersed throughout the book are blank pages, presumably for the reader's convenience for extra notes or drawings.

The book may be highly recommended as an outline or primer on the subject of fractures.

G. O. E.

The Man Who Lived for Tomorrow. A Biography of William Hallock Park, M.D. By WADE W. OLIVER. 507 pp. $3.75. *E. P. Dutton & Co., New York, 1941.*

This is more than a biography of Park. It is an interesting historical account of the part played by the bacteriological laboratory in revolutionizing public health practice during the last half-century. For Park's laboratory, more than any other, mirrored this age of discovery and progress in the control of human infections. From 1893, when he accepted appointment as the "Bacteriological Diagnostician and Inspector of Diphtheria" in the New York City Health Department, until his retirement in 1935 as Director of its Bureau of Laboratories, he and a brilliant devoted group of fellow workers produced the greatest body of constructive research directly applicable to solution of problems in community health that has ever emanated from any municipal laboratory in the world. Into the background of these activities, obscured too often by unimportant detail, is woven the theme of Park's life. For those who knew him, for those who are interested in bacteriology, or in public health, or in preventive medicine, something valuable will be found between these two covers.

K. F. M.

Age Morphology of Primary Tubercles. By HENRY C. SWEANY. 265 pp. Illus. $5.00. *Charles C. Thomas, Springfield, Illinois, 1941.*

This is a monograph based on the study of the pathological anatomy of tuberculosis with special reference to the relationship of the morphology of primary tubercles to their age. The theory is held that encapsulation and restoration of injured tissues proceed at a fairly definite rate and that the pathologist can tell the age of primary tubercles from their appearance. Interesting correlations are made between history of known contact and the presumptive age of the primary tubercles found on pathological examination.

The clinician and roentgenologist will regret that the author has not attempted to present the serial roentgenological observations of these patients, many of whom were known to be tuberculous for a number of years before their death, and whose chest films in series might have thrown light upon the author's interesting theory. The material from autopsies has been studied with great care and the book is well written and abundantly illustrated.

M. B.

Neuro-Ophthalmology. By R. LINDSAY REA. 2nd ed. 688 pp. Illus. $13.50. *The C. V. Mosby Company, St. Louis, 1941.*

The second edition of this text-book is larger than the first edition by 111 pages of factual material which has been included under the title, "Appendix to Second Edition." This is a misnomer since almost all the added material supplies information which amplifies that contained in the first volume. Furthermore, the 516 pages of text in the original edition have not been changed in any way whatsoever.

An individual who owns the first edition, and who buys this present volume is paying a very high price ($13.50) for 111 pages of text and a few additional references. Without doubt the publishers have already received many complaints on this score, and in the opinion of this reviewer, such complaints are well founded.

After having made this criticism it is only fair to state that the book is an extremely useful one. The author has succeeded in presenting a reasonably complete description of a majority of those conditions which are important both from the neurological and ophthalmological points of view.

F. W.

Principal Nervous Pathways. By ANDREW THEODORE RASMUSSEN. 2nd Ed. 73 pp. Illus. $2.50. *The Macmillan Co., New York 1941.*

The author of this handy reference book is an enthusiastic teacher of Neurology. He has attempted to circumscribe that enthusiasm in the outlines of the nervous pathways which he has drawn for his own students.

In the second edition a few minor revisions have brought the outlines and descriptive text up to date, particularly those of the central connections of the second and of the eighth nerves. The paper and the type have been changed, giving a crisper looking page. The change in the spacing of text on the page is less pleasing than that found in the first edition. The drawings are clear and concise, easily giving the illusion of the third dimension. Some of these outlines are unique, particularly those of the peripheral distribution of the visceral afferent and efferent systems. These in particular will be useful to the medical student in his clinical years and to the practitioner who has not specialized in the nervous system.

M. H.

Infant Nutrition. By WILLIAMS MCKIM MARRIOTT. Revised by P. C. JEANS. 3rd ed. 475 pp. Illus. $5.50. *C. V. Mosby Company, St. Louis, 1941.*

The new edition of Marriott's text, revised by one of his former associates, is a welcome addition to our current literature. The concise style and critical viewpoint which won for this volume a wide popularity has been retained in the present revision.

In comparing this volume with its predecessors one finds relatively few changes. Recent developments in the vitamin field have been introduced as well as some material on chemotherapy. There are new growth charts and a chapter on allergy has been added. The reviewer regrets, however, that the excellent discussion of Marriott on anhydremia, acidosis and alkalosis has been

considerably abridged. On the whole, the work of revision has been well done and deserves commendation.

L. E. H., Jr.

The American Illustrated Medical Dictionary. By W. A. Newman Dorland. 19th ed. 1647 pp. Illus. $7.00. Thumb-indexed, $7.50. *W. B. Saunders Company, Philadelphia and London, 1941.*

This, the nineteenth edition of Dorland's dictionary, is of course quite similar to previous editions, but the preface states that 2,000 new words have been added. This rather depressing thought of the increase in medical terminology is ameliorated somewhat when one notes that in the preface to the previous edition, the eighteenth, it was announced that over 3,000 new words had been defined.

S. V. L.

Taber's Cyclopedic Medical Dictionary. By Clarence Wilbur Taber and Associates. 1488 pp. Illus. Thumb-indexed $3.00. Plain $2.50. *F. A Davis Company, Philadelphia, 1940.*

A new dictionary in a field in which there are already a number of excellent examples needs must have some special reasons for its being. This dictionary seeks to fulfill this condition by being more than a dictionary. Thus for many terms there is given, in addition to the definition, a brief description of the condition, symptoms, treatment, and sometimes nursing procedure. In other words, it may be called a practical encyclopedia. This would seem to be a rather doubtful advantage.

S. V. L.

Diagnosis and Treatment of Diseases of the Peripheral Arteries. By Saul S. Samuels. 2nd ed. 372 pp. Illus. $6.50. *Oxford University Press, London, New York, Toronto, 1940.*

The author has revised his book and added brief chapters dealing with acrocyanosis, periarteritis nodosa, embolism, cervical ribs and scalenus anticus syndrome, thrombosis of the axillary vein, frostbite, ergotism, glomus tumors and aneurysm of the peripheral vessels. The new edition otherwise has the same virtues and deficiencies as the original. The diagnosis of peripheral arterial disorders is discussed in a clear and helpful manner. The conservative treatment of obliterative arterial diseases, and especially the use of intravenous hypertonic saline solution, is presented with enthusiasm and the results cited are excellent. To those, however, who have failed to find such treatment equally helpful, it will seem that the method in general fails to measure up to the efficacy suggested by the author's personal series. Likewise, to those who have found that other methods, and particularly sympathetic ganglionectomy, frequently bring about brilliant and dramatic results, often in cases which have done badly on good medical treatment, the author will seem to have neglected some of the most valuable aids in treatment of these disorders.

H. B. S., Jr.

The Heart in Pregnancy and the Childbearing Age. By BURTON E. HAMILTON and K. JEFFERSON THOMSON. 402 pp. Illus. $5.00. *Little, Brown and Company, Boston, 1941.*

This book is an intensely practical survey of the whole problem of heart disease in pregnancy based on the extensive experience of the Boston Lying-In Hospital during the past twenty years. Throughout this period the senior author has been cardiologist to that hospital, where his pioneer work has not only revised the prognosis of the gravida with heart disease but has altered the attitude of obstetricians and internists alike to the problem. The volume is written in an exceptionally straight-forward, conversational style—much as if a master clinician, out of the fullness of his experience and hard-won wisdom, were telling *extempore* of the lessons he had learned. This makes the book both readable and convincing.

The work is divided into three parts. The first portion deals with the practical aspects of the care of cardiac patients throughout pregnancy, delivery and puerperium and contains important information for any general practitioner, obstetrician or internist who shares in their care. A valuable section on the obstetrical care of patients with cardiac disease is contributed by Dr. Frederick C. Irving, Obstetrician-in-Chief to the Boston Lying-In Hospital. The second part of the book concerns itself with the physiology of the circulation during gestation both in normal women and in those with heart disease. It sets forth clearly and convincingly the underlying rationale of our modern handling of cardiac conditions in pregnancy. Finally, a section is devoted to a discussion of the various heart diseases in women of the childbearing age and the effect of gestation upon the course of the disease.

In sum, this seems to the reviewer to be an excellent book which should be read by every physician who has to do with heart disease in pregnancy.

N. J. E.

A Textbook of Bacteriology. By R. W. FAIRBROTHER. 3rd ed. 451 pp. Illus. $5.00. *C. V. Mosby Company, St. Louis, 1941.*

It is hard to know what to say about this book and, as it is already in its third edition, any criticism now seems immaterial. Written primarily for medical students, "A Textbook of Bacteriology" makes no pretense of being an exhaustive treatise on the subject. It is simply written; its explanations are clear and easy to follow; its text is not cluttered up. Inevitably, however, it has the defects which correspond to these virtues and the more sophisticated bacteriologist cannot help being struck by what has been sacrificed for the sake of simplicity and clarity. The most glaring deficiency is the total lack of bibliographical material. The investigators who were responsible for the major developments in bacteriology are mentioned by name, but there is not a single reference to the literature in the entire book.

E. A. B.

BOOKS RECEIVED FOR REVIEW

Textbook of General Surgery. By WARREN H. COLE and ROBERT ELMAN. 3rd ed. 1067 pp. Illus. $8.00. *D. Appleton-Century Company, New York, 1941.*

The Universal Constant in Living. By F. MATTHIAS ALEXANDER. 270 pp. $2.50. *E. P. Dutton & Co., New York, 1941.*

The Man Who Lived for Tomorrow. A Biography of William Hallock Park, M.D. By WADE W. OLIVER. 507 pp. $3.75. *E. P. Dutton & Co., New York, 1941.*

The Art and Science of Nutrition. By ESTELLE E. HAWLEY and GRACE CARDEN. 619 pp. Illus. $3.50. *C. V. Mosby Company, St. Louis, 1941.*

Professional Adjustments. I. By GENE HARRISON. 204 pp. $2.25. *C. V. Mosby Company, St. Louis, 1941.*

Synopsis of the Preparation and Aftercare of Surgical Patients. By HUGH C. ILGENFRITZ and RAWLEY M. PENICK, JR., with foreword by URBAN MAES. 532 pp. Illus. $5.00. *C. V. Mosby Company, St. Louis, 1941.*

Immunization to Typhoid Fever. American Journal of Hygiene Monographic Series, No. 17, September, 1941. 276 pp. Illus. $2.50. *The Johns Hopkins Press, Baltimore, 1941.*

Cancer of the Face and Mouth. By BILRAY P. BLAIR, SHERWOOD MOORE, and LOUIS T. BYARS. 599 pp. Illus. $10.00. *C. V. Mosby Company, St. Louis, 1941.*

The Retina. By S. L. POLYAK. 607 pp. Illus. $10.00. *University of Chicago Press, Chicago, 1941.*

Modern Treatment of Syphilis. By JOSEPH EARLE MOORE. 2nd ed. 674 pp. Illus. $7.00. *Charles C. Thomas, Springfield, Illinois, 1941.*

America's Last King. By MANFRED S. GUTTMACHER. 426 pp. Illus. $3.50. *Charles Scribner's Sons, New York, 1941.*

Diseases of Women. By HARRY STURGEON CROSSEN and ROBERT JAMES CROSSEN. 9th ed. 948 pp. Illus. $12.50. *C. V. Mosby Company, St. Louis, 1941.*

From Cretin to Genius. By SERGE VORONOFF. 281 pp. $2.75. *Alliance Book Corporation, New York, 1941.*

Infant Nutrition. By WILLIAMS MCKIM MARRIOTT. Revised by P. C. Jeans. 3rd ed. 475 pp. Illus. $5.50. *C. V. Mosby Company, St. Louis, 1941.*

Sinus. By RUSSELL CLARK GROVE. 165 pp. Illus. $2.00. *Alfred A. Knopf, New York, 1941.*

Occupational Diseases. By RUTHERFORD T. JOHNSTONE. 558 pp. Illus. $7.50. *W. B. Saunders Company, Philadelphia & London, 1941.*

William Henry Welch and the Heroic Age of American Medicine. By SIMON FLEXNER and JAMES THOMAS FLEXNER. 539 pp. Illus. $3.75. *Viking Press, New York, 1941.*